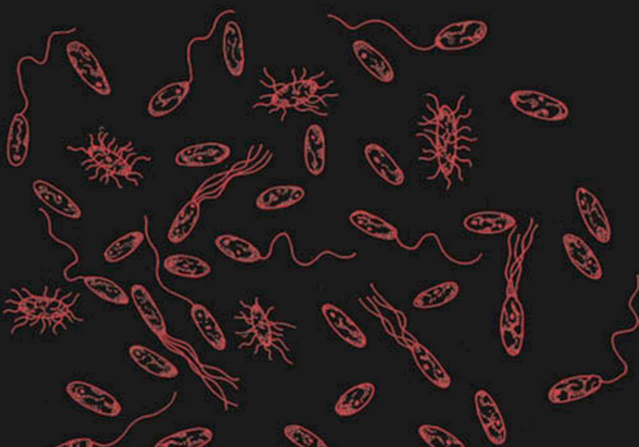
Wastewater Microbiology Series

The Microbiology of Anaerobic Digesters



MICHAEL H. GERARDI

The Microbiology of Anaerobic Digesters

WASTEWATER MICROBIOLOGY SERIES

Editor

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Nitrification and Denitrification in the Activated Sludge Process
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To
Mom and Dad

The author extends his sincere appreciation to
joVanna Gerardi for computer support
and
Cristopher Noviello for artwork used in this text.

Contents

Preface

Completely mixed anaerobic digesters are the most commonly used treatment system in North America for the degradation of municipal sludges. Although these suspended-growth systems are not used as commonly at industrial wastewater treatment plants, more and more industrial plants are using fixed-film anaerobic digesters for the treatment of soluble organic compounds in their wastewaters.

Anaerobic digesters perform most of the degradation of organic compounds at wastewater treatment plants. However, digesters often experience operational problems that result in process upsets and increased operational costs. Examples of process upsets and operational problems include foam and scum production, decanting and dewatering difficulties, loss of treatment efficiency, toxic upsets, and "souring" of the digester. Poorly operating anaerobic digesters often contribute to operational problems in other treatment units such as the activated sludge process, gravity thickener, clarifiers, and sludge dewatering facilities.

Because of the importance of anaerobic digesters in wastewater treatment processes, a review of the microbiology of the bacteria and the operational conditions that affect their activity is of value in addressing successful and cost-effective operation. This book provides an in-depth review of the bacteria, their activity, and the operational conditions that affect anaerobic digester performance. The identification of operational problems and troubleshooting and corrective measures for process control are presented.

This book is prepared for an audience of operators and technicians who are responsible for the daily operation of anaerobic digesters. It presents troubleshooting and process control measures to reduce operational costs, maintain treatment efficiency, and prevent system upsets.

The Microbiology of Anaerobic Digesters is the third book in the Wastewater Microbiology Series by John Wiley & Sons. This series is designed for operators and technicians, and it presents a microbiological review of the organisms involved in wastewater treatment processes and provides biological techniques for monitoring and regulating these processes.

Michael H. Gerardi
Linden, Pennsylvania

Part I

Overview

1

Introduction

The organic content of sludges and soluble wastes can be reduced by controlled bacterial activity. If the bacterial activity is anaerobic, the reduction in organic content is achieved through sludge digestion. If the bacterial activity is aerobic, the reduction in organic content is achieved through sludge stabilization.

Anaerobic digesters having suspended bacterial growth are commonly used at municipal wastewater treatment plants to degrade (digest) sludges (Figure 1.1). With the development of anaerobic digesters having fixed-film bacterial growth (Figure 1.2), more and more industrial wastewater treatment plants are using anaerobic digesters to degrade soluble organic wastes. Anaerobic digesters represent catabolic (destructive) processes that occur in the absence of free molecular oxygen (O_2).

The goals of anaerobic digesters are to biologically destroy a significant portion of the volatile solids in sludge and to minimize the putrescibility of sludge. The main products of anaerobic digesters are biogas and innocuous digested sludge solids. Biogas consists mostly of methane (CH_4) and carbon dioxide (CO_2).

Primary and secondary sludges are degraded in anaerobic digesters (Figure 1.3). Primary sludge consists of the settled solids from primary clarifiers and any colloidal wastes associated with the solids. Secondary sludge consists mostly of waste-activated sludge or the humus from trickling filters. The mixture of primary and secondary sludges contains 60% to 80% organic matter (dry weight) in the forms of carbohydrates, fats, and proteins.

The mixture of primary and secondary sludges is an ideal medium for bacterial growth. The sludges are rich in substrates (food) and nutrients and contain a large number and diversity of bacteria required for anaerobic digestion.

The anaerobic digester is well known as a treatment process for sludges that contain large amounts of solids (particulate and colloidal wastes). These solids

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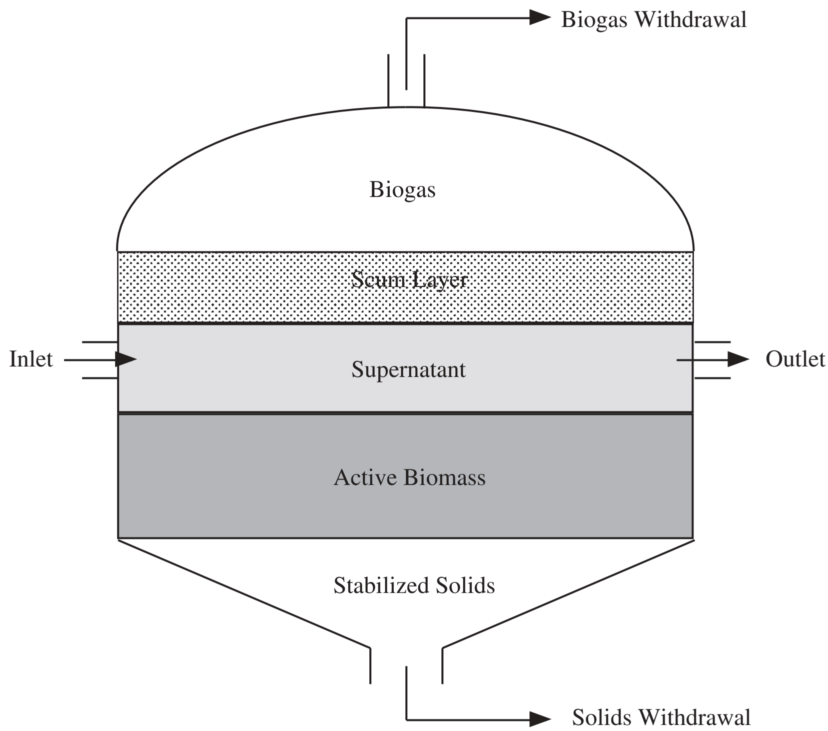


Figure 1.1 Suspended growth anaerobic digesters are commonly used at municipal wastewater treatment plants for the degradation of primary and secondary sludges. These digesters produce several layers as a result of sludge degradation. These layers are from top to bottom: biogas, scum, supernatant, active biomass or sludge, and stabilized solids.

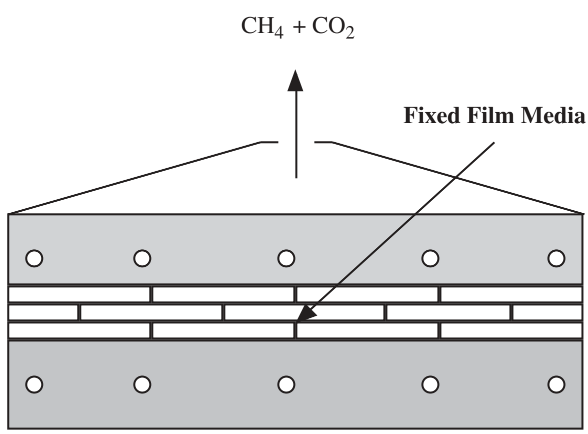


Figure 1.2 Fixed film anaerobic digesters employ the use of a medium such as plastic or rocks on which bacteria grow as a biofilm. Wastewater passing over the medium is absorbed and adsorbed by the biofilm and degraded.

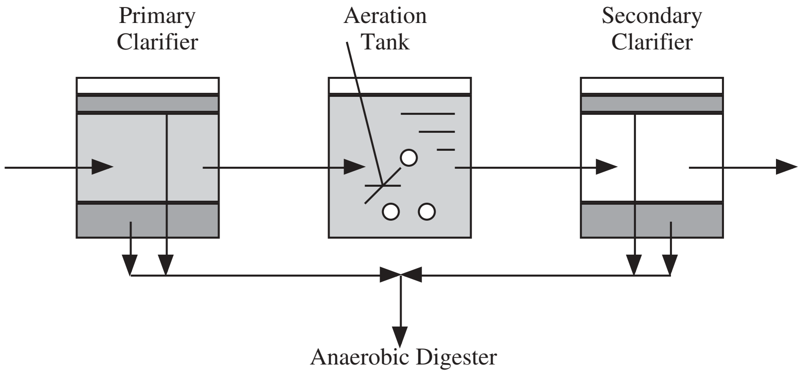


Figure 1.3 *Primary and secondary sludges typically are degraded in suspended growth anaerobic digesters at municipal wastewater treatment plants. The sludges contain relatively large quantities of particulate and colloidal wastes.*

require relatively long digestion periods (10–20 days) to allow for the slow bacterial processes of hydrolysis and solubilization of the solids. Once solubilized, the resulting complex organic compounds are degraded to simplistic organic compounds, mostly volatile acids and alcohols, methane, new bacterial cells ($C_5H_7O_2N$), and a variety of simplistic inorganic compounds such as carbon dioxide and hydrogen gas (H_2).

With the development of fixed-film bacterial growth in anaerobic digesters, many soluble organic wastes can be digested quickly and efficiently. Because the wastes are soluble, time is not required for hydrolysis and solubilization of the wastes.

When sludges are digested, the organic content of the sludges is decreased as volatile materials within the sludges are destroyed, that is, the volume and weight of the solids are reduced. The volatile content for most anaerobic digested sludges is 45%–55% (Figure 1.4).

Anaerobic digesters (Figure 1.5) degrade approximately 80% of the influent organic waste of a conventional municipal wastewater treatment plant. Nearly 30% of the waste is removed by primary clarifiers and transferred to anaerobic digesters, and approximately 50% of the waste is synthesized or transformed into new bacterial cells or solids [mixed-liquor volatile suspended solids (MLVSS) or trickling filter humus]. These synthesized solids also are transferred to anaerobic digesters through the wasting of secondary solids.

Because of the relatively large quantity of organic wastes placed on the anaerobic digestion process, a review of the bacteria, their activity, and the operational factors that influence their activity are critical. This review provides for proper maintenance of digester performance and cost-effective operation and helps to ensure adequate monitoring, troubleshooting, and process control of anaerobic digesters.

Anaerobic sludge digestion consists of a series of bacterial events that convert organic compounds to methane, carbon dioxide, and new bacterial cells. These events are commonly considered as a three-stage process.

The first stage of the process involves the hydrolysis of solids (particulate and colloidal wastes). The hydrolysis of these wastes results in the production of

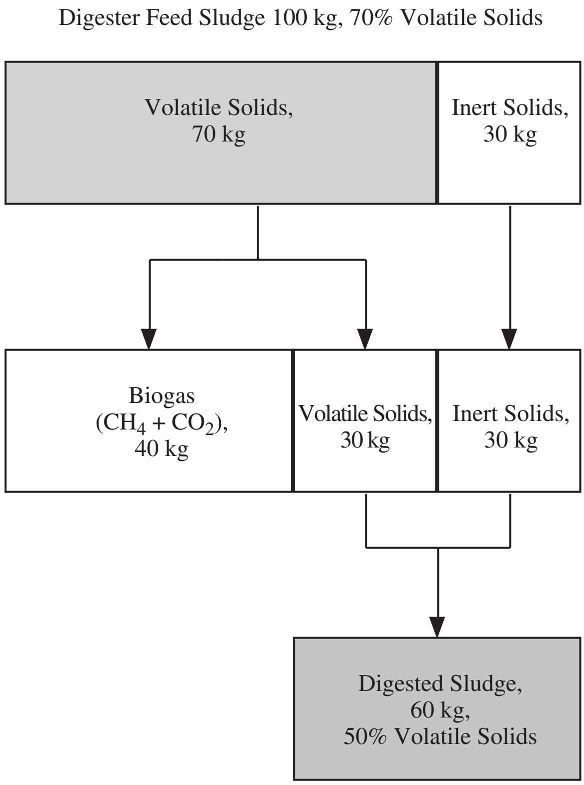


Figure 1.4 *The digestion of sludges in anaerobic digesters results in significant reduction in the volatile content of the sludges as well as the volume and weight of the sludges.*

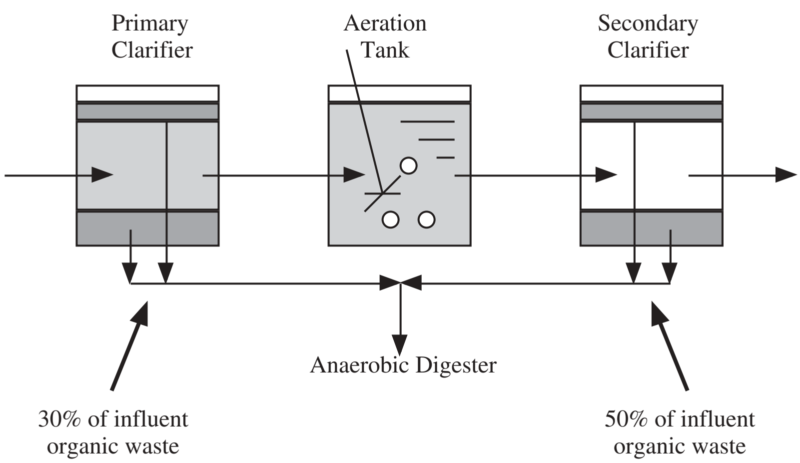


Figure 1.5 *Most of the influent organic wastes of a wastewater treatment plant are degraded in an anaerobic digester. Settled solids in the primary clarifier represent approximately 30% of the influent organic wastes, while secondary solids represent approximately 50% of the influent organic wastes. In the activated sludge process much of the organic waste is converted to bacterial cells. These cells represent organic wastes, i.e., upon their death; they serve as a substrate for surviving bacteria.*

simplistic, soluble organic compounds (volatile acids and alcohols). The second stage of the process, acetogenesis, involves the conversion of the volatile acids and alcohols to substrates such as acetic acid or acetate (CH_3COOH) and hydrogen gas that can be used by methane-forming bacteria. The third and final stage of the process, methanogenesis, involves the production of methane and carbon dioxide.

Hydrolysis is the solubilization of particulate organic compounds such as cellulose (Equation 1.1) and colloidal organic compounds such as proteins (Equation 1.2) into simple soluble compounds that can be absorbed by bacterial cells. Once absorbed, these compounds undergo bacterial degradation that results in the production of volatile acids and alcohols such as ethanol (CH_3CH_2OH) and propionate (CH_3CH_2COOH). The volatile acids are converted to acetate and hydrogen gas. Methane production occurs from the degradation of acetate (Equation 1.3) and the reduction of carbon dioxide by hydrogen gas (Equation 1.4).

$$\text{cellulose} + H_2O \text{ —hydrolysis} \rightarrow \text{soluble sugars} \tag{1.1}$$

$$\text{proteins} + H_2O \text{ —hydrolysis} \rightarrow \text{soluble amino acids} \tag{1.2}$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{1.3}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{1.4}$$

In addition to the reduction in volume and weight of sludges, anaerobic digesters provide many attractive features including decreased sludge handling and disposal costs and reductions in numbers of pathogens (Table 1.1). The relatively high temperatures and long detention times of anaerobic digesters significantly reduce the numbers of viruses, pathogenic bacteria and fungi, and parasitic worms. This reduction in numbers of pathogens is an extremely attractive feature in light of the increased attention given by regulatory agencies and the general public with respect to health risks represented by the use of digested sludges (biosolids) for agricultural and land reclamation purposes.

Although anaerobic digesters offer many attractive features, anaerobic digestion of sludges unfortunately has an unwarranted reputation as an unstable and difficult-to-control process. This unwarranted reputation is due to several reasons, including a lack of adequate knowledge of anaerobic digester microbiology and proper operational data (Table 1.2).

TABLE 1.1 Attractive Features of Anaerobic Digesters

Able to degrade recalcitrant natural compounds, e.g., lignin
Able to degrade xenobiotic compounds, e.g., chlorinated aliphatic hydrocarbons
Control of some filamentous organisms through recycling of sludge and supernatant
Improved dewaterability of sludge
Production of methane
Use of biosolids as a soil additive or conditioner
Suitable for high-strength industrial wastewater
Reduction in malodors
Reduction in numbers of pathogens
Reduction in sludge handling and disposal costs
Reduction in volatile content of sludge

TABLE 1.2 Reasons Contributing to the Unwarranted Reputation of the Anaerobic Digester as an Unstable Process

Lack of adequate knowledge of anaerobic digester microbiology
Lack of commercial interest
Lack of operator training
Lack of proper operational performance data for installed digesters
Lack of research and academic status
Regrowth needed for industrial toxicity episodes

TABLE 1.3 Examples of Significant Differences Between Aerobic Stabilization and Anaerobic Digestion of Wastes

Feature	Anaerobic Digestion	Aerobic Stabilization
Process rate	Slower	Faster
Sensitivity to toxicants	Higher	Lower
Start-up time	Slower	Faster

Until recently, little information was available that reviewed the bacteria and their requirements for anaerobic digestion of solids. The difficulty in obtaining adequate data was caused by the overall complex anaerobic digestion process, the very slow generation time of methane-forming bacteria, and the extreme "sensitivity" of methane-forming bacteria to oxygen. Therefore, it was not uncommon for operators to have problems with digester performance.

These problems, the development and use of aerobic "digesters," and the use of relatively cheap energy for aerobic stabilization of wastes contributed to the lack of interest in anaerobic digesters. Although aerobic stabilization, that is, the use of aerobic digesters, and anaerobic digestion of wastes are commonly used at wastewater treatment process, significant differences exist between these biological processes (Table 1.3).

Methane production under anaerobic conditions has been occurring naturally for millions of years in such diverse habitats as benthic deposits, hot springs, deep ocean trenches, and the intestinal tract of cattle, pigs, termites, and humans. Methane production also occurs in rice paddies.

More than 100 years ago, anaerobic digesters were first used in Vesoul, France to degrade domestic sludge. Until recently, anaerobic digesters were used mostly to degrade municipal sludges and food-processing wastewater. Municipal sludges and food-processing wastewater favor the use of anaerobic digesters, because the sludges and wastewater contain a large diversity of easily degradable organics and a large complement of inorganics that provide adequate nutrients and alkalinity that are needed in the anaerobic digestion process.

TABLE 1.4 Chemical Wastes Amenable to Anaerobic Digestion

Acetone	Formate
Acrylates	Glycerol
Alcohols	Glycols
Aldehydes	Ketones
Amino acids	Methyl acetate
Anilines	Nitrobenzene
Catechols	Organic acids
Cresol	Phenols
Formaldehyde	Quinones

TABLE 1.5 Industrial Wastes Amenable to Anaerobic Digestion

Alcohol stillage	Pectin
Bean	Petroleum
Beverage production	Pharmaceutical
Brewery	Potato
Canning	Pulp and paper
Cheese	Seafood and shellfish
Chemical	Slaughterhouse and meat packing
Corn	Sugar
Dairy	Vegetable
Distillery	Wheat and grain
Egg	Winery
Fruit	Wool scouring
Leachate	Yeast

A better understanding of the microbiology of anaerobic digesters and process modifications, particularly fixed-film processes, have permitted the use of anaerobic digesters for dilute wastewaters and a large variety of industrial wastes (Tables 1.4 and 1.5). This understanding and these process modifications, together with the need to pretreat industrial wastewaters and sludges and the attractive features of anaerobic digesters, have generated renewed interest in their use in degrading not only municipal sludges but also industrial wastewaters.

The number of wastes that are amenable to anaerobic digestion is quite large. Examples of industrial wastes include acetone, butanol, cresol, ethanol, ethyl acetate, formaldehyde, formate, glutamate, glycerol, isopropanol, methanol, methyl acetate, nitrobenzene, pentanol, phenol, propanol, isopropyl alcohol, sorbic acid, *tert*-butanol, and vinyl acetate. Because many industrial wastes can be treated anaerobically, the feasibility of anaerobic digestion of an industrial waste is determined by several factors. These factors include the concentration of the waste, the temperature of the waste stream, the presence of toxicants, biogas and sludge production, and expected treatment efficiency.

The development of the fixed film filter was a significant achievement in anaerobic technology (Figure 1.6). The filter provides relatively long solids retention time (SRT). Increased retention time makes it possible to treat moderately low-strength

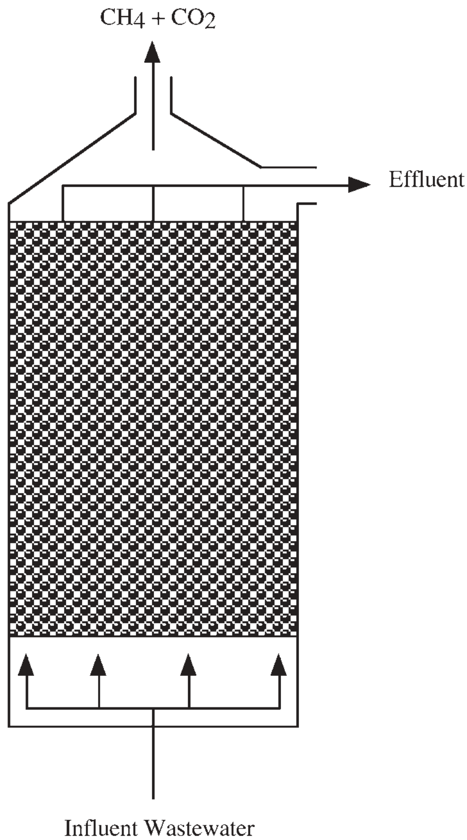


Figure 1.6 *In an anaerobic filter, wastewater flows from bottom to top or top to bottom of the treatment unit. The wastewater passes over media that contains a fixed film of bacteria growth that degrades the organic wastes in the wastewater.*

[2000–20,000 mg/l chemical oxygen demand (COD)] soluble organic industrial waste. Because of the highly concentrated bacterial population of the filter, a highly stable digestion process can be achieved even during significant variations in operating conditions and loadings. Therefore, interest in anaerobic biotechnology for treating industrial waste streams has grown considerably.

2

Bacteria

At least 300 different species of bacteria are found in the feces of a single individual. Most of these bacteria are strict anaerobes. The majority of the remaining bacteria are facultative anaerobes. *Escherichia coli* is a common facultative anaerobe in feces.

Bacteria from fecal wastes as well as hundreds of soil and water bacteria that enter a conveyance system through inflow and infiltration (I/I) are found in the influent of municipal wastewater treatment processes. For the purpose of this text, bacteria that are commonly found in wastewater treatment processes are divided into groups according to 1) their response to free molecular oxygen (O_2) and 2) their enzymatic ability to degrade substrate in the anaerobic digester.

RESPONSE TO FREE MOLECULAR OXYGEN

Bacteria may be divided further into three groups according to their response to free molecular oxygen (Table 2.1). These groups are 1) strict aerobes, 2) facultative anaerobes, and 3) anaerobes, including the methane-forming bacteria.

Strict aerobes are active and degrade substrate only in the presence of free molecular oxygen. These organisms are present in relatively large numbers in aerobic fixed-film processes, for example, trickling filters, and aerobic suspended-growth processes, for example, activated sludge. In the presence of free molecular oxygen they perform significant roles in the degradation of wastes. However, strict aerobes die in an anaerobic digester in which free molecular oxygen is absent.

Facultative anaerobes are active in the presence or absence of free molecular oxygen. If present, free molecular oxygen is used for enzymatic activity and the

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TABLE 2.1 Groups of Bacteria According to Their Response to Free Molecular Oxygen

Group	Example	Significance
Strict aerobes	*Haliscomenobacter hydrossis*	Degrades soluble organic compounds; contributes to filamentous sludge bulking
	Nitrobacter sp.	Oxidizes NO_2^- to NO_3^-
	Nitrosomonas sp.	Oxidizes NH_4^+ to NO_2^-
	Sphaerotilus natans	Degrades soluble organic compounds; contributes to filamentous sludge bulking
	Zoogloea ramigera	Degrades soluble organic compounds; contributes to floc formation
Facultative anaerobes	*Escherichia coli*	Degrades soluble organic compounds; contributes to floc formation; contributes to denitrification or clumping
	Bacillus sp.	Degrades soluble organic compounds; contributes to denitrification or clumping
Anaerobes	*Desulfovibrio* sp.	Reduces SO_4^{2-} to H_2S
	Methanobacterium formicium	Produces CH_4

TABLE 2.2 Groups of Anaerobic Bacteria

Group	Example	Significance
Oxygen tolerant	*Desulfovibrio* sp.	Reduces SO_4^{2-} to H_2S
	Desulfomarculum sp.	Reduces SO_4^{2-} to H_2S
Oxygen intolerant	*Methanobacterium formicium*	Produces CH_4
	Methanobacterium propionicium	Produces CH_4

degradation of wastes. If free molecular oxygen is absent, another molecule, for example, nitrate ion (NO_3^-), is used to degrade wastes such as methanol (CH_3OH) (Equation 2.1). When nitrate ions are used, denitrification occurs and dinitrogen gas (N_2) is produced.

$$6NO_3^- + 5CH_3OH \rightarrow 2N_2 + 5CO_2 + 7H_2O + 6OH^- \tag{2.1}$$

Most bacteria within fixed-film processes and suspended growth processes are facultative anaerobes, and these organisms also perform many significant roles in the degradation of wastes. Approximately 80% of the bacteria within these aerobic processes are facultative anaerobes. These organisms are found in relatively large numbers not only in aerobic processes but also in anaerobic processes.

During the degradation of wastes within an anaerobic digester, facultative anaerobic bacteria, for example, *Enterobacter* spp., produce a variety of acids and alcohols, carbon dioxide (CO_2), and hydrogen from carbohydrates, lipids, and proteins. Some organisms, for example, *Escherichia coli*, produce malodorous compounds such as indole and skatole.

Anaerobes are inactive in the presence of free molecular oxygen and may be divided into two subgroups: oxygen-tolerant species and oxygen-intolerant species or strict anaerobes (Table 2.2). Some anaerobes are strong acid producers, such as, *Streptococcus* spp., whereas other anaerobes, such as *Desulfomarculum* spp., reduce sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S) (Equation 2.2). Although oxygen-tolerant anaerobes survive in the presence of free molecular oxygen, these organisms cannot

perform normal cellular activities, including the degradation of substrate, in the presence of free molecular oxygen. Strict anaerobes, including methane-forming bacteria, die in the presence of free molecular oxygen.

$$CH_3COOH + SO_4^{2-} \rightarrow 2CO_2 + 2H_2O + H_2S \quad (2.2)$$

Numerous acid-forming bacteria are associated with methane-forming bacteria. These organisms include facultative anaerobes that ferment simple, soluble organic compounds and strict anaerobes that ferment complex proteins and carbohydrates.

The products of fermentation vary greatly depending on the bacteria involved in the fermentative process. Therefore, changes in operational conditions that result in changes in dominant bacteria also result in changes in the concentrations of acids and alcohols that are produced during fermentation. Changes in the concentrations of acids and alcohols significantly change the substrates available for methane-forming bacteria, their activity, and, consequently, digester performance.

Most strict anaerobes are scavengers. These organisms are found where anaerobic conditions exist in lakes, river bottoms, human intestinal tracts, and anaerobic digesters. Anaerobes survive and degrade substrate most efficiently when the oxidation-reduction potential (ORP) of their environment is between –200 and –400 millivolts (mV). Any amount of dissolved oxygen in an anaerobic digester raises the ORP of the sludge and discourages anaerobic activity including hydrolysis, acetogenesis, and methanogenesis. Therefore, sludges and wastewaters fed to an anaerobic digester should have no molecular oxygen. Settled and thickened sludges usually do not have a residual dissolved oxygen concentration. These sludges typically have a low ORP (–100 to –300 mV).

The ORP of a wastewater or sludge can be obtained by using an electrometric pH meter with a millivolt scale and an ORP probe. The ORP of a wastewater or sludge is measured on the millivolt scale of the pH meter.

The ORP is a measurement of the relative amounts of oxidized materials, such as nitrate ions (NO_3^-) and sulfate ions (SO_4^{2-}), and reduced materials, such as ammonium ions (NH_4^+) (Table 2.3). At ORP values greater than +50 mV, free molecular oxygen is available in the wastewater or sludge and may be used by aerobes and facultative anaerobes for the degradation of organic compounds. This degradation occurs under an oxic condition.

At ORP values between +50 and –50 mV, free molecular oxygen is not available but nitrate ions or nitrite ions (NO_2^-) are available for the degradation of organic compounds. The degradation of organic compounds without free molecular oxygen is an anaerobic condition. The use of nitrate ions or nitrite ions occurs under an anoxic condition and is referred to as denitrification, clumping, and rising sludge in the secondary clarifier of an activated sludge process.

At ORP values less than –50 mV, nitrate ions and nitrite ions are not available but sulfate ions are available for the degradation of organic compounds. This degradation also occurs without free molecular oxygen. When sulfate is used to degrade organic compounds, sulfate is reduced and hydrogen sulfide is formed along with a variety of acids and alcohols.

At ORP values less than –100 mV, the degradation of organic compounds proceeds as one portion of the compound is reduced while another portion of the compound is oxidized. This form of anaerobic degradation of organic compounds is

TABLE 2.3 Oxidation-reduction Potential (ORP) and Cellular Activity

Approximate ORP, mV	Carrier Molecule for Degradation of Organic Compounds	Condition	Respiration
>+50	O_2	Oxic	Aerobic
+50 to –50	NO_3^- or NO_2^-	Anaerobic	Anoxic
<–50	SO_4^{2-}	Anaerobic	Fermentation, sulfate reduction
<–100	Organic Compound	Anaerobic	Fermentation, mixed acid production
<–300	CO_2	Anaerobic	Fermentation, methane production

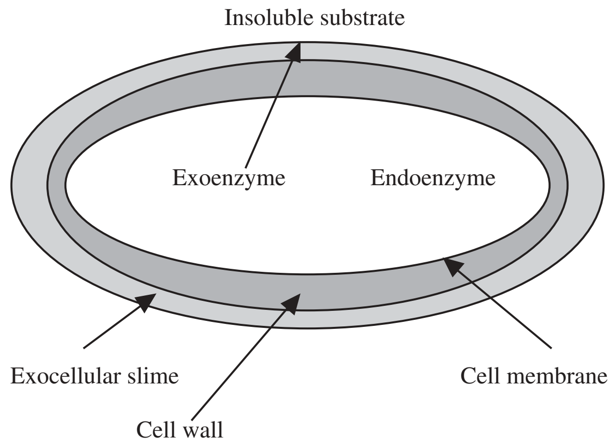


Figure 2.1 *There are two types of enzymes that are used by bacteria to degrade substrate. Exoenzymes are produced in the cell and released through the cell membrane and cell wall to hydrolyze insoluble substrate that is adsorbed to the exocellular slime. Soluble wastes enter the bacterial cell and are degraded by endoenzymes.*

commonly known as mixed-acid fermentation because a mixture of acids, for example, acetate, butyrate, formate, and propionate, are produced. A mixture of alcohols is also produced during fermentation.

At ORP values less than –300 mV, anaerobic degradation of organic compounds and methane production occur. During methane production, simple organic compounds such as acetate are converted to methane, and carbon dioxide and hydrogen are combined to form methane.

ENZYMATIC ABILITY TO DEGRADE SUBSTRATE

Bacteria degrade substrate through the use of enzymes. Enzymes are proteinaceous molecules that catalyze biochemical reactions. Two types of enzymes are involved in substrate degradation—endoenzymes and exoenzymes (Figure 2.1).

Endoenzymes are produced in the cell and degrade soluble substrate within the cell. Exoenzymes also are produced in the cell but are released through the "slime" coating the cell to the insoluble substrate attached to the slime. Once in contact with the substrate the exoenzyme solubilizes particulate and colloidal substrates. Once

TABLE 2.4 Exoenzymes and Substrates

Substrate to be Degraded	Exoenzyme Needed	Example	Bacterium	Product
Polysaccharides	Saccharolytic	Cellulase	*Cellulomonas*	Simple sugar
Proteins	Proteolytic	Protease	*Bacillus*	Amino acids
Lipids	Lipolytic	Lipase	*Mycobacterium*	Fatty acids

solubilized, these substrates enter the cell and are degraded by endoenzymes. The production of exoenzymes and solubilization of particulate and colloidal substrates usually take several hours.

All bacteria produce endoenzymes, but not all bacteria produce exoenzymes. No bacterium produces all the exoenzymes that are needed to degrade the large variety of particulate and colloidal substrates that are found in sludges and wastewaters (Table 2.4). Each exoenzyme as well as each endoenzyme degrades only a specific substrate or group of substrates. Therefore, a large and diverse community of bacteria is needed to ensure that the proper types of exoenzymes and endoenzymes are available for degradation of the substrates present.

The relative abundance of bacteria within an anaerobic digester often is greater than 10^{16} cells per milliliter. This population consists of saccharolytic bacteria (~10^{8} cells/ml), proteolytic bacteria (~10^{6} cells/ml), lipolytic bacteria (~10^{5} cells/ml), and methane-forming bacteria (~10^{8} cells/ml).

There are three important bacterial groups in anaerobic digesters with respect to the substrates utilized by each group. These groups include the acetate-forming (acetogenic) bacteria, the sulfate-reducing bacteria, and the methane-forming bacteria. The acetate-forming bacteria and sulfate-reducing bacteria are reviewed in this chapter, and the methane-forming bacteria are reviewed in Chapter 3.

ACETATE-FORMING BACTERIA

Acetate-forming (acetogenic) bacteria grow in a symbiotic relationship with methane-forming bacteria. Acetate serves as a substrate for methane-forming bacteria. For example, when ethanol (CH_3CH_2OH) is converted to acetate, carbon dioxide is used and acetate and hydrogen are produced (Equation 2.3).

$$CH_3CH_2OH + CO_2 \rightarrow CH_3COOH + 2H_2 \quad (2.3)$$

When acetate-forming bacteria produce acetate, hydrogen also is produced. If the hydrogen accumulates and significant hydrogen pressure occurs, the pressure results in termination of activity of acetate-forming bacteria and lost of acetate production. However, methane-forming bacteria utilize hydrogen in the production of methane (Equation 2.4) and significant hydrogen pressure does not occur.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.4)$$

Acetate-forming bacteria are obligate hydrogen producers and survive only at very low concentrations of hydrogen in the environment. They can only survive if their metabolic waste—hydrogen—is continuously removed. This is achieved in

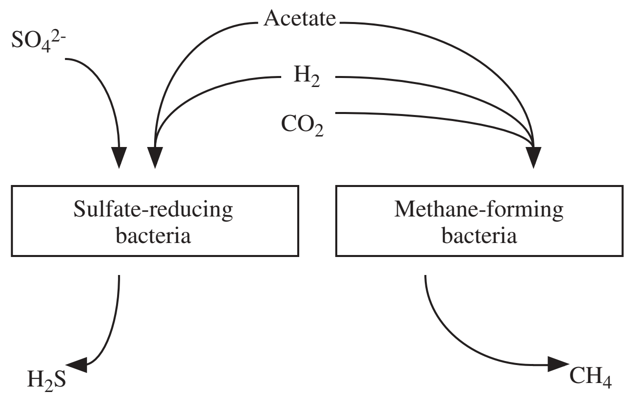


Figure 2.2 *Many different groups of bacteria within the anaerobic digester often compete for the same substrate and electron acceptor. An example of this competition is the used of acetate and hydrogen by sulfate-reducing bacteria and methane-forming bacteria. Acetate is used by as a substrate by both groups of bacteria. Methane is produced by methane-forming bacteria and a variety of acids and alcohols are produced by sulfate reducing bacteria. Hydrogen is used with sulfate (SO_4^{2-}) by sulfate-reducing bacteria and hydrogen sulfide (H_2S) is produced.*

their symbiotic relationship with hydrogen-utilizing bacteria or methane-forming bacteria. Acetogenic bacteria reproduce very slowly. Generation time for these organisms is usually greater than 3 days.

SULFATE-REDUCING BACTERIA

Sulfate-reducing bacteria also are found in anaerobic digesters along with acetate-forming bacteria and methane-forming bacteria. If sulfates are present, sulfate-reducing bacteria such as *Desulfovibrio desulfuricans* multiply. Their multiplication or reproduction often requires the use of hydrogen and acetate—the same substrates used by methane-forming bacteria (Figure 2.2).

When sulfate is used to degrade an organic compound, sulfate is reduced to hydrogen sulfide. Hydrogen is needed to reduce sulfate to hydrogen sulfide. The need for hydrogen results in competition for hydrogen between two bacterial groups, sulfate-reducing bacteria and methane-producing bacteria.

When sulfate-reducing bacteria and methane-producing bacteria compete for hydrogen and acetate, sulfate-reducing bacteria obtain hydrogen and acetate more easily than methane-forming bacteria under low-acetate concentrations. At substrate-to-sulfate ratios <2, sulfate-reducing bacteria out-compete methane-forming bacteria for acetate. At substrate-to-sulfate ratios between 2 and 3, competition is very intense between the two bacterial groups. At substrate-to-sulfate ratios >3, methane-forming bacteria are favored.

The hydrogen sulfide produced by sulfate-reducing bacteria has a greater inhibitory effect at low concentrations on methane-forming bacteria and acetate-forming bacteria than on acid-forming bacteria.

3

Methane-forming Bacteria

Methane-forming bacteria are known by several names (Table 3.1) and are a morphologically diverse group of organisms that have many shapes, growth patterns, and sizes. The bacteria can be found as individual rods, curved rods, spirals, and cocci (Figure 3.1) or grouped as irregular clusters of cells, chains of cells or filaments, and sarcina or cuboid arrangements (Figure 3.2). The range in diameter sizes of individual cells is 0.1–15 µm. Filaments can be up to 200 µm in length. Motile and nonmotile bacteria (Figure 3.3) as well as spore-forming and non-spore-forming bacteria can be found.

Methane-forming bacteria are some of the oldest bacteria and are grouped in the domain Archaebacteria (from *arachae* meaning "ancient") (Figure 3.4). The domain thrives in heat. Archaebacteria comprise all known methane-forming bacteria, the extremely halophilic bacteria, thermoacidophilic bacteria, and the extremely thermophilic bacteria. However, the methane-forming bacteria are different from all other bacteria.

Methane-forming bacteria are oxygen-sensitive, fastidious anaerobes and are free-living terrestrial and aquatic organisms. Although methane-forming bacteria are oxygen sensitive, this is not a significant disadvantage. Methane-forming bacteria are found in habitats that are rich in degradable organic compounds. In these habitats, oxygen is rapidly removed through microbial activity. Many occur as symbionts in animal digestive tracts. Methane-forming bacteria also have an unusually high sulfur content: Approximately 2.5% of the total dry weight of the cell is sulfur.

The of methane-forming bacteria are classified in the domain Archaebacteria because of several unique characteristics that are not found in the true bacteria or Eubacteria. These features include 1) a "nonrigid" cell wall and unique cell membrane lipid, 2) substrate degradation that produces methane as a waste, and 3)

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TABLE 3.1 Commonly Used Names for Methane-forming Bacteria

Methanogenic bacteria
Methanogens
Methane-forming bacteria
Methane-producing bacteria

specialized coenzymes. The cell wall lacks muramic acid, and the cell membrane does not contains an ether lipid as its major constituent (Figure 3.5). Coenzymes that are unique to methane-forming bacteria are coenzyme M and the nickel-containing coenzymes F_{420} and F_{430}. Coenzyme M is used to reduce carbon dioxide (CO_2) to methane. The nickel-containing coenzymes are important hydrogen carriers in methane-forming bacteria.

The coenzymes are metal laden organic acids that are incorporated into enzymes and allow the enzymes to work more efficiently. The coenzymes are components of energy-producing electron transfer systems that obtain energy for the bacterial cell and remove electrons from degraded substrate (Figure 3.6).

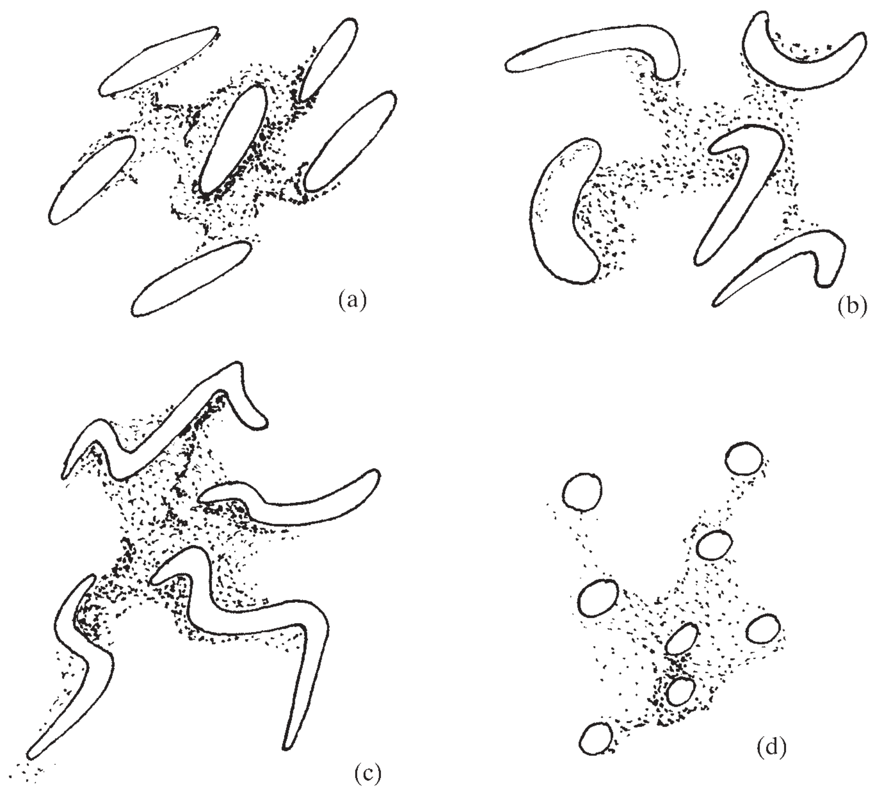


Figure 3.1 Common shapes of methane-forming bacterial cells. Commonly occurring shapes of methane-forming bacteria include rod or bacillus (a), curved rod (b), spiral (c), and coccus or spherical (d).

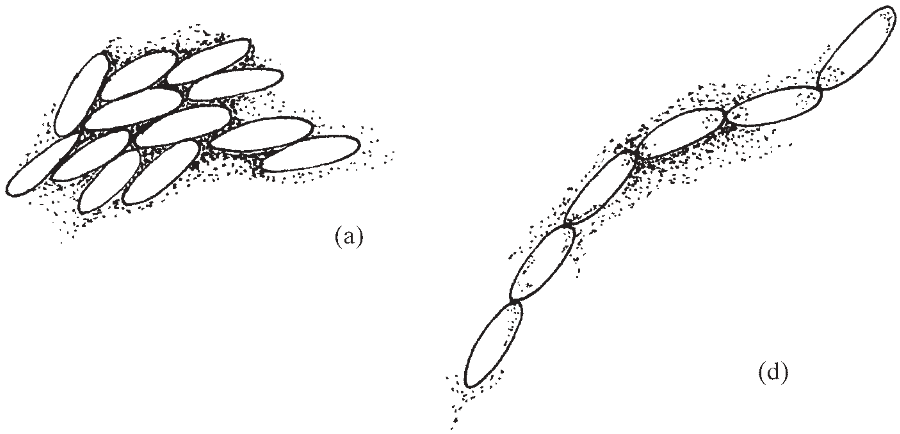


Figure 3.2 Common growth patterns of methane-forming bacterial cells. Commonly occurring growth patterns of methane-forming bacteria include an irregular cluster (a) and a filamentous chain (b).

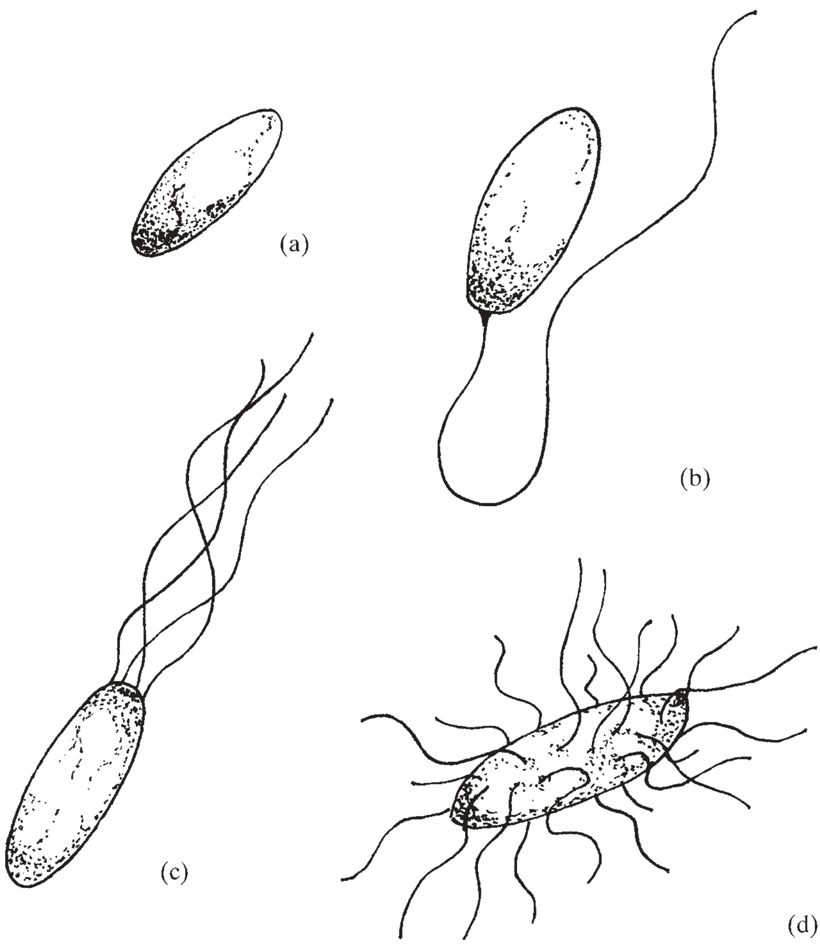


Figure 3.3 Non-motile and motile methane-forming bacteria. Methane-forming bacteria may be non-motile (a) or motile (b, c, and d). Motile bacteria possess a flagellum or several flagella. The flagellum or flagella may be located at one end of the cell or on the entire surface of the bacterial cell.

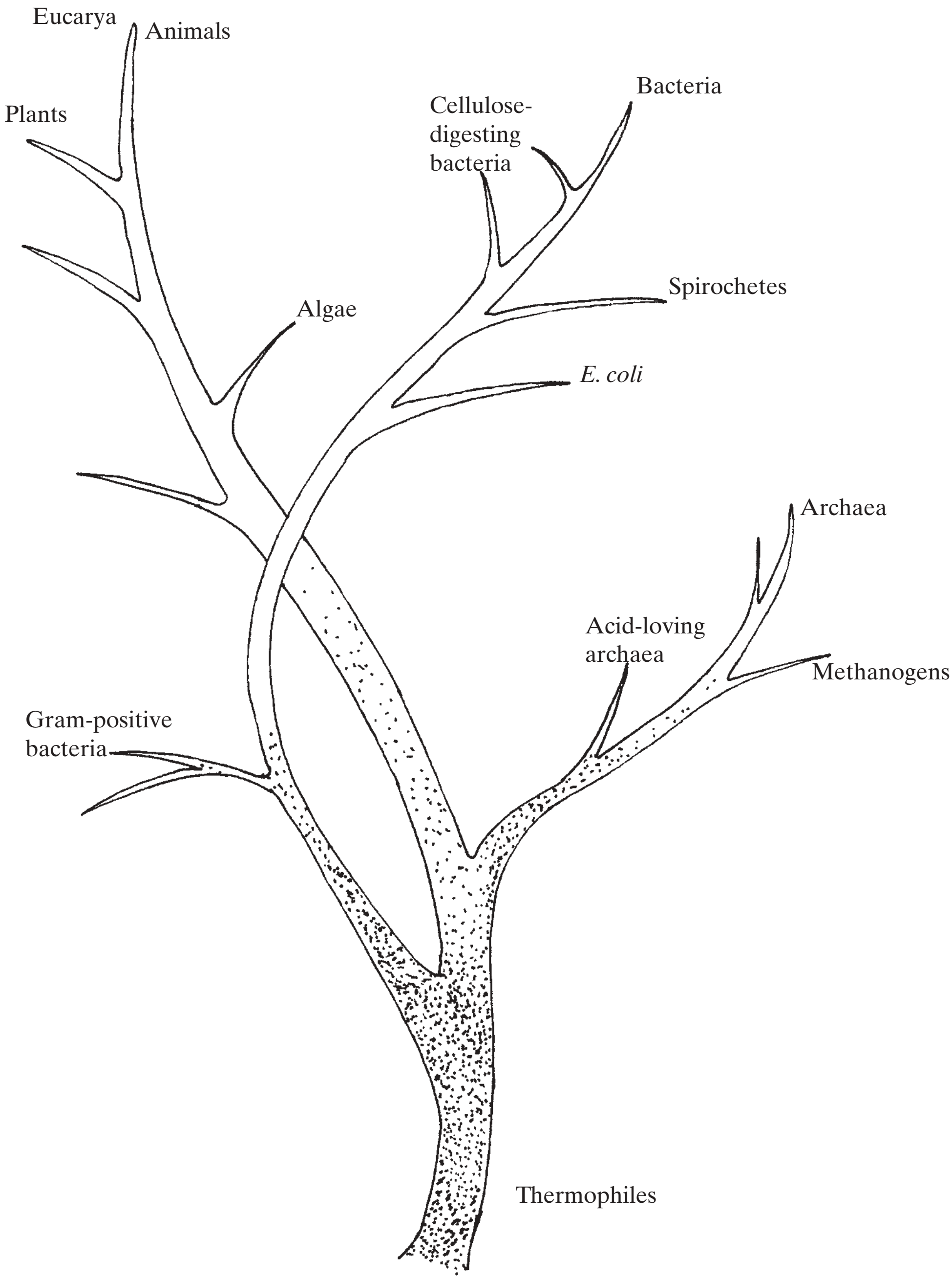
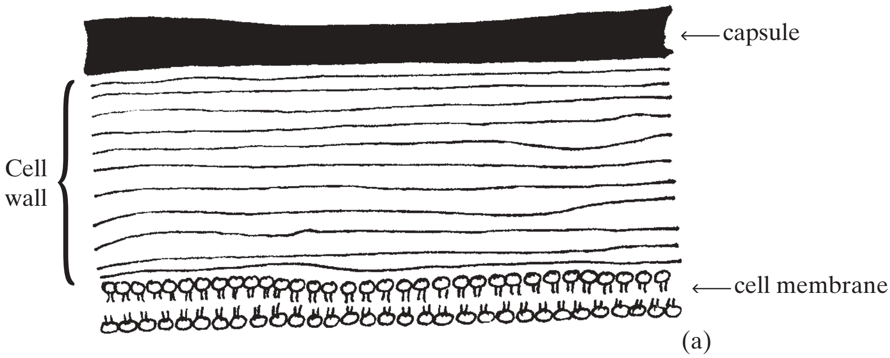


Figure 3.4 *Location of methane-forming bacteria on the phylogenetic tree. The phylogenetic tree (the historical development of different life forms) contains old (*arachae*) life forms closest to the base of the tree, while new life forms closest to the end of the branches. The tree contains the domains Thermopiles, Archaea, Eubacteria (true bacteria), and the Eucarya (higher life forms). The methane-forming bacteria are found closest to the base of the tree.*



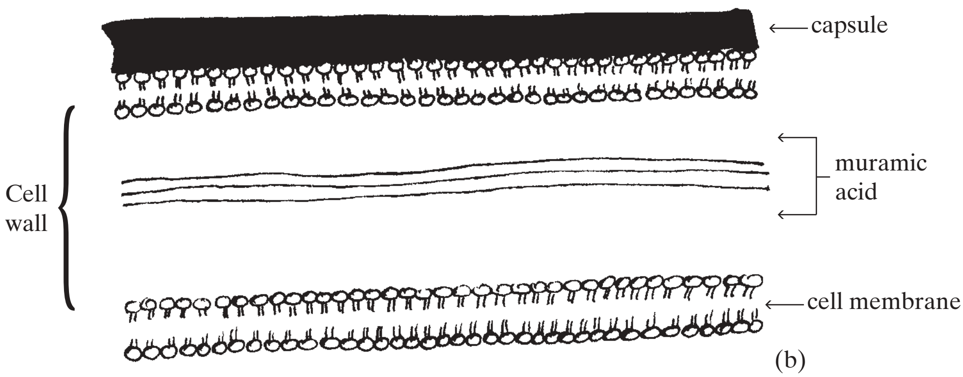


Figure 3.5 *Cell wall of methane-forming bacteria. The cell wall of methane-forming bacteria (a) does not contain muramic acid, while the cell of other bacteria (b) contains varying amounts of muramic acid.*

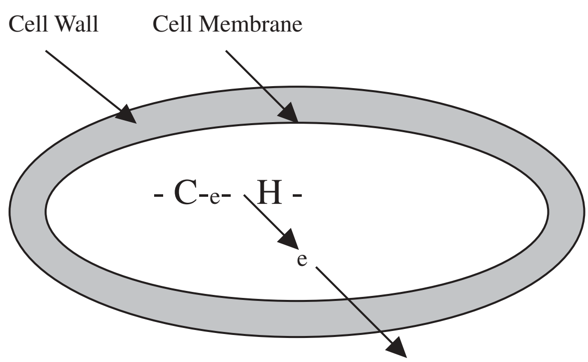


Figure 3.6 *Electrons (e) released from broken chemical bonds of substrates inside a bacterial cell are removed through the used of electron transport systems. These systems employ the use of proteins that contain co-enzymes such as metals and vitamins.*

TABLE 3.2 Examples of Methane-forming Bacteria with and without a Protective Envelope

Genus	Envelope
Methanobacterium	Absent
Methanobrevibacter	Absent
Methanosarcina	Absent
Methanococcus	Present
Methanogenium	Present
Methanomicrobium	Present
Methanospirillum	Present

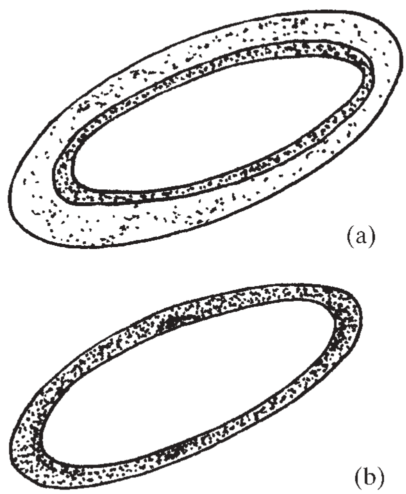


Figure 3.7 *Presence of an envelope on some methane-forming bacteria. Some methane-forming bacteria possess an envelope (a) that provides added protection for the bacterial cell. Methane-forming bacteria that do not possess an envelope (b) are easily lyzed in the presence of surfactants.*

The unique chemical composition of the cell wall makes the bacteria "sensitive" to toxicity from several fatty acids. Also, many methane-forming bacteria lack a protective envelope around their cell wall (Table 3.2). Surfactants or hypotonic shock easily lyse methane-forming bacteria that do not have this envelope (Figure 3.7).

All methane-forming bacteria produce methane. No other organism produces methane. Methane-forming bacteria obtain energy by reducing simplistic compounds or substrates such as carbon dioxide and acetate (CH_3COOH). Some methane-forming bacteria are capable of fixing molecular nitrogen (N_2).

Methane-forming bacteria are classified according to their structure, substrate utilization, types of enzymes produced, and temperature range of growth. There are approximately 50 species of methane-forming bacteria that are classified in three orders and four families (Table 3.3).

Methane-forming bacteria grow as microbial consortia, tolerate high concentrations of salt, and are obligate anaerobes. The bacteria grow on a limited number of

TABLE 3.3 Groups of Methane-forming Bacteria

Order	Family
Methanobacteriales	Methanobacteriaceases
Methanococcales	Methanococcaceae
Methanomicrobials	Methanomicrobiaceas
	Methanosarcinaceae

substrates. *Methanobacterium formicium*, for example, grows on formate, carbon dioxide, and hydrogen and is one of the more abundant methane-forming bacteria in anaerobic digesters. *Methanobacterium formicium* performs a significant role in sludge digestion and methane production. *Methanobacterium formicium* and *Methanobrevibacter arboriphilus* are two of the dominant methane-forming bacteria in anaerobic digesters. The activity of these organisms and that of all methane-forming bacteria is usually determined by measuring changes in volatile acid concentration or methane production.

In nature, methane-forming bacteria perform two very special roles. They participate in the degradation of many organic compounds that are considered biorecalcitrant, that is, can only be degraded slowly, and they produce methane from the degradation of organic compounds. Methane is poorly soluble in water, inert under anaerobic conditions, non-toxic, and able to escape from the anaerobic environment.

Methane-forming bacteria are predominantly terrestrial and aquatic organisms and are found naturally in decaying organic matter, deep-sea volcanic vents, deep sediment, geothermal springs, and the black mud of lakes and swamps. These bacteria also are found in the digestive tract of humans and animals, particularly the rumen of herbivores and cecum of non-ruminant animals.

The rumen is a special organ in the digestive tract in which the degradation of cellulose and complex polysaccharides occurs. Cows, goats, sheep, and deer are examples of ruminant animals. The bacteria, including methane-forming bacteria, that grow in the digestive tract of ruminant animals are symbionts and obtain most of their carbon and energy from the degradation of cellulose and other complex polysaccharides from plants. Ruminants cannot survive without the bacteria. The bacteria and substrates produced by the bacteria through their fermentative activities provide the ruminants with most of their carbon and energy.

Methane-forming bacteria grow well in aquatic environments in which a strict anaerobic condition exists. The anaerobic condition of an aquatic environment is expressed in terms of its oxidation-reduction potential or ORP (Table 3.4). Methane-forming bacteria grow best in an environment with an ORP of less than –300 mV. Most facultative anaerobes do well in aquatic environments with an ORP between +200 and –200 mV.

There are Gram-negative and Gram-positive methane-forming bacteria that reproduce slowly. Gram stain results (negative, positive, and variable) are different within the same order of methane-forming bacteria because of their different types of cell walls (Figure 3.8).

The reproductive times or generation times for methane-forming bacteria range from 3 days at 35°C to 50 days at 10°C. Because of the long generation time of methane-forming bacteria, high retention times are required in an anaerobic digester to ensure the growth of a large population of methane-forming bacteria for

TABLE 3.4 Oxidation-Reduction Potential (ORP) and Cellular Activity

Approximate ORP Values, mV	Molecule Used for Degradation of Substrate	Type of Degradation or Respiration
>+50	Oxygen (O_2)	Oxic (aerobic)
+50 to –50	Nitrite (NO_2^-) or nitrate (NO_3^-)	Anoxic (anaerobic)
<–50	Sulfate (SO_4^{2-})	Sulfate reduction (anaerobic)
<–100	Organic (CHO)	Fermentation (mixed acids and alcohol production)
<–300	Organic (CHO), CO_2, CO, H_2	Fermentation (methane production)

Order of Reagent	Reagent	Color of Gram-positive Bacteria	Color of Gram-negative Bacteria
Primary Stain	Crystal Violet	Violet	Violet
Mordant	Iodine	Violet	Violet
Decoloring Agent	95% Alcohol	Violet	Colorless
Counter Stain	Safranin	Violet	Red

Figure 3.8 *Gram staining is a laboratory technique that separates bacteria into two grous, Gram-positive and Gram-negative, depending on the response of bacteria to the stains Crystal violet and Safranin. The technique was developed in 1884 by the Danish bacteriologist Christian Gram. Although the technique was developed as a procedure for detecting pathogenic bacteria, it is used for taxonomic (classification) and identification purposes.*

The response of bacteria to the Gram stain is determined by microscopic examination of bacteria that have been successively stained with a basic dye (Crystal violet), treated with an iodine solution or mordant, and rinsed with an organic solvent such as acetone or alcohol. Gram-positive bacteria retain the violet stain and are violet under microscopic examination. Gram-negative bacteria are decolorized by the solvent. The colorless, Gram-negative bacteria are stained with the counter stain Safranin to impart a pink or red color.

the degradation of organic compounds. At least 12 days are required to obtain a large population of methane-forming bacteria.

Methane-forming bacteria obtain their energy for reproduction and cellular activity from the degradation of a relatively small number of simple substrates (Table 3.5). These substrates include hydrogen, 1-carbon compounds, and acetate as the 2-carbon compound. One-carbon compounds include formate, methanol, carbon dioxide, carbon monoxide (CO), and methylamine. The most familiar and frequently acknowledged substrates of methane-forming bacteria are acetate and hydrogen. Acetate is commonly split to form methane while hydrogen is combined with carbon dioxide to form methane. The splitting of acetate to form methane is known as aceticlastic cleavage.

Each methane-forming bacterium has a specific substrate or group of substrates that it can degrade (Table 3.6). Hydrogen can serve as a universal substrate for

TABLE 3.5 Substrates Used by Methane-forming Bacteria

Substrate	Chemical Formula
Acetate	CH_3COOH
Carbon dioxide	CO_2
Carbon monoxide	CO
Formate	$HCOOH$
Hydrogen	H_2
Methanol	CH_3OH
Methylamine	CH_3NH_2

TABLE 3.6 Species of Methane-forming Bacteria and Their Substrates

Species	Substrate
Methanobacterium formicium	Carbon dioxide, formate, hydrogen
Methanobacterium thermoantotrophicum	Hydrogen, carbon dioxide, carbon monoxide
Methanococcus frisius	Hydrogen, methanol, methylamine
Methanococcus mazei	Acetate, methanol, methylamine
Methanosarcina bakerii	Acetate, carbon dioxide, hydrogen, methanol, methylamine

methane-forming bacteria, and carbon dioxide functions as an inorganic carbon source in the forms of carbonate (CO_3^{2-}) or bicarbonate (HCO_3^-). Carbon dioxide also serves as a terminal acceptor of electrons released by degraded substrate.

Other 1-carbon compounds that can be converted to substrates for methane-forming bacteria include dimethyl sulfide, dimethylamine, and trimethylamine. Several alcohols including 2-propanol and 2-butanol as well as propanol and butanol may be used in the reduction of carbon dioxide to methane.

The majority of methane produced in an anaerobic digester occurs from the use of acetate and hydrogen by methane-forming bacteria. The fermentation of substrates such as acetate (aceticlastic cleavage) results in the production of methane (Equation 3.1), and the reduction of carbon dioxide also results in the production of methane (Equation 3.2).

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{3.1}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{3.2}$$

Aceticlastic cleavage of acetate and reduction of carbon dioxide are the two major pathways to methane production. Fermentation of propionate (CH_3CH_2COOH) and butyrate ($CH_3CH_2CH_2COOH$) are minor pathways to methane production. However, the fermentation of propionic acid to methane requires two different species of bacteria and two microbial degradation steps (Equations 3.3 and 3.4). In the first reaction, methane and acetate are produced from the fermentation of propionate by a volatile acid-forming bacterium (*Syntrophobacter wolinii*) and a methane-forming bacterium. In the second reaction, methane is produced from the cleavage of acetate by a methane-forming bacterium. These reactions occur only if hydrogen and formate are kept low (used) by

methane-forming bacteria. Accordingly, the accumulation of propionate is a common indicator of stress in an anaerobic digester.

$$4CH_3CH_2COOH + 2H_2O \rightarrow 4CH_3COOH + CO_2 + 3CH_4 \quad (3.3)$$

$$4CH_3COOH \rightarrow 4CH_4 + 4CO_2 \quad (3.4)$$

Butyrate also is degraded to methane through two microbial degradation steps (Equations 3.5 and 3.6). The degradation steps again are mediated by two different bacteria. In the first reaction, methane and acetate are produced from the fermentation of butyrate by a volatile acid-forming bacterium and a methane-forming bacterium. In the second reaction, methane is produced from the cleavage of acetate by a methane-forming bacterium. Because butyrate can be used indirectly by methane-forming bacteria, its accumulation is an indicator of stress in an anaerobic digester.

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 4CH_3COOH + CO_2 + CH_4 \quad (3.5)$$

$$4CH_3COOH \rightarrow 4CH_4 + 4CO_2 \quad (3.6)$$

No species of methane-forming bacteria can utilize all substrates. Therefore, successful fermentation of substrates in an anaerobic digester requires the presence of not only a large number of methane-forming bacteria but also a large diversity of methane-forming bacteria.

There are three principal groups of methane-forming bacteria. These groups are 1) the hydrogenotrophic methanogens, 2) the acetotrophic methanogens, and 3) the methylotrophic methanogens. The term "trophic" (from *trophē*, "nourishment") refers to the substrates used by the bacteria.

GROUP 1 HYDROGENOTROPHIC METHANOGENS

The hydrogenotrophic methanogens use hydrogen to convert carbon dioxide to methane (Equation 3.7). By converting carbon dioxide to methane, these organisms help to maintain a low partial hydrogen pressure in an anaerobic digester that is required for acetogenic bacteria.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (3.7)$$

GROUP 2 ACETOTROPHIC METHANOGENS

The acetotrophic methanogens "split" acetate into methane and carbon dioxide (Equation 3.8). The carbon dioxide produced from acetate may be converted by hydrogenotrophic methanogens to methane (Equation 3.7). Some hydrogenotrophic methanogens use carbon monoxide to produce methane (Equation 3.9).

$$4CH_3COOH \rightarrow 4CO_2 + 2H_2 \quad (3.8)$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \quad (3.9)$$

The acetotrophic methanogens reproduce more slowly than the hydrogenotrophic methanogens and are adversely affected by the accumulation of hydrogen. Therefore, the maintenance of a low partial hydrogen pressure in an anaerobic digester is favorable for the activity of not only acetate-forming bacteria but also acetotrophic methanogens. Under a relatively high hydrogen partial pressure, acetate and methane production are reduced.

GROUP 3 METHYLOTROPHIC METHANOGENS

The methylotrophic methanogens grow on substrates that contain the methyl group ($-CH_3$). Examples of these substrates include methanol (CH_3OH) (Equation 3.10) and methylamines [$(CH_3)_3-N$] (Equation 3.11). Group 1 and 2 methanogens produce methane from CO_2 and H_2. Group 3 methanogens produce methane directly from methyl groups and not from CO_2.

$$3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O \tag{3.10}$$

$$4(CH_3)_3 - N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \tag{3.11}$$

The use of different substrates by methane-forming bacteria results in different energy gains by the bacteria. For example, hydrogen-consuming methane production results in more energy gain for methane-forming bacteria than acetate degradation. Although methane production using hydrogen is the more effective process of energy capture by methane-forming bacteria, less than 30% of the methane produced in an anaerobic digester is by this method. Approximately 70% of the methane produced in an anaerobic digester is derived from acetate. The reason for this is the limited supply of hydrogen in an anaerobic digester. The majority of methane obtained from acetate is produced by two genera of acetotrophic methanogens, *Methanosarcina* and *Methanothrix*.

Reproduction of methane-forming bacteria is mostly by fission, budding, constriction, and fragmentation (Figure 3.9). Methane-forming bacteria reproduce very slowly. This slow growth rate is due to the relatively small amount of energy obtained from the use of their limited number of substrates. Therefore, a relatively large quantity of substrates must be fermented for the population of methane-forming bacteria to double, that is, a relatively small quantity of cells or sludge is produced for a relatively large quantity of substrate degraded. Therefore, anaerobic digesters produce relatively small quantities of bacteria cells or sludge (solids).

Under optimal conditions, the range of generation times of methane-forming bacteria may be from a few days to several weeks. Therefore, if solids retention time (SRT) is short or short-circuiting or early withdrawal of digester sludge occurs, the population size of methane-forming bacteria is greatly reduced. These conditions decrease the time available for reproduction of methane-forming bacteria, that is, the bacteria are removed from the digester faster than they can reproduce. This results in poor digester performance or failure of the digester.

With increasing retention time the production of new methane-forming bacteria gradually decreases as a result of increased energy requirements of the cells in order

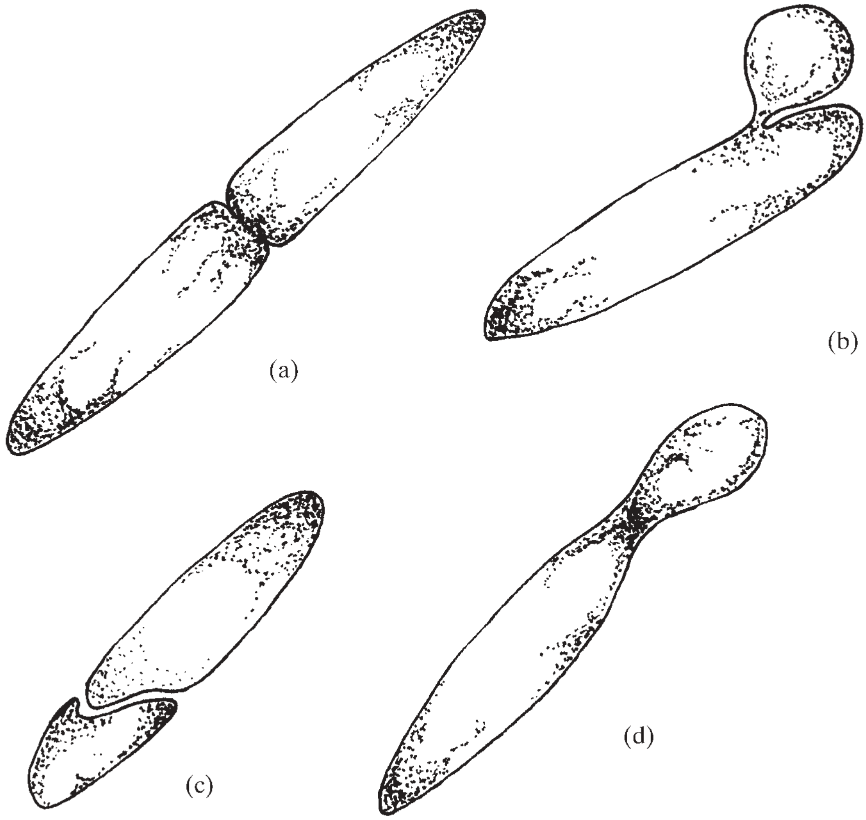


Figure 3.9 *Modes of reproduction for methane-forming bacteria. Methane-forming bacteria reproduce very slowly. Generation time for these organisms is usually greater than 3 days. Reproduction is asexual and may occur through fission (a), budding (b), fragmentation (c), and constriction (d).*

TABLE 3.7 Optimal Growth Temperature of Some Methane-forming Bacteria

Genus	Temperature Range, °C
Methanobacterium	37–45
Methanobrevibacter	37–40
Methanosphaera	35–40
Methanothermus	83–88
Methanococcus	35–40
	65–91
Methanocorpusculum	30–40
Methanoculleus	35–40
Methanogenium	20–40
Methanoplanus	30–40
Methanospirillum	35–40
Methanococcoides	30–35
Methanohalobium	50–55
Methanohalophilus	35–45
Methanolobus	35–40
Methanosarcina	30–40
	50–55
Methanothrix	35–50

to maintain cellular activity (more degradation of substrate). Therefore, increasing retention time of a properly operated anaerobic digester results in decreased sludge production. Increasing retention time produces a large consumption of substrate by slowing reproducing bacteria as an energy requirement of old cells (sludge) for the maintenance of cellular activity.

Most methane-forming bacteria are mesophiles or thermophiles, with some bacteria growing at temperatures above 100°C (Table 3.7). Mesophiles are those organisms that grow best within the temperature range of 30–35°C, and thermophiles are those organisms that grow best within the temperature range of 50–60°C. Some genera of methane-forming bacteria have mesophilic and thermophilic species.

It is difficult to grow methane-forming bacteria in pure culture. Standard laboratory enumeration techniques are not suitable for methane-forming bacteria. This difficulty is caused by 1) their extreme obligate anaerobic nature and the probability that they are killed rapidly by relatively short time exposures to air compared with other anaerobes and 2) their limited number of substrates. To correct for the oxygen sensitivity of methane-forming bacteria in laboratory experiments with pure cultures, the "Hungate" technique is used. Growth or cell masses of methane-forming bacteria may be gray, green, greenish black, orange-brown, pink, purple, yellow, or white.

4

Respiration

Respiration is one of many cellular processes. For the purpose of this text, respiration is considered to be the degradation of substrate to obtain cellular nourishment. During respiration large compounds of high energy content are broken down to small compounds of low energy content (Figure 4.1). Much of the energy lost by the large compounds is captured by the respiring organisms. This capture results in a gain in the amount of useful energy.

Two types of nourishment are obtained from the degradation of substrate—carbon and energy. Carbon is required for the synthesis of cellular materials for growth and reproduction. Energy is required for cellular activity including reproduction. Bacteria may obtain their nourishment from one substrate or two substrates. The energy substrate may be organic or inorganic.

Most bacteria use organic compounds to obtain carbon and energy. These organisms are known as organotrophs. The term "troph" comes from the Greek *trophē*, meaning "nourishment." Organotrophs obtain their carbon and energy from the degradation of organic compounds such as glucose ($C_6H_{12}O_6$). An example of an organotroph is *Zoogloea ramigera*. This bacterium is a floc former that degrades soluble organic compounds in the activated sludge and trickling filter processes. Another example of an organotroph is *Pseudomonas aeruginosa*. This bacterium degrades soluble organic compounds in activated sludge and trickling filter processes and anaerobic digesters.

Some bacteria use inorganic compounds to obtain carbon and energy. These organisms are known as chemoautotrophs. They obtain their carbon from carbon dioxide (CO_2) and their energy from inorganic compounds. An example of a chemoautotroph is *Nitrobacter winogradski*. This bacterium oxidizes nitrite ions (NO_2^-) to nitrate ions (NO_2^-) to obtain energy and uses carbon dioxide in the form

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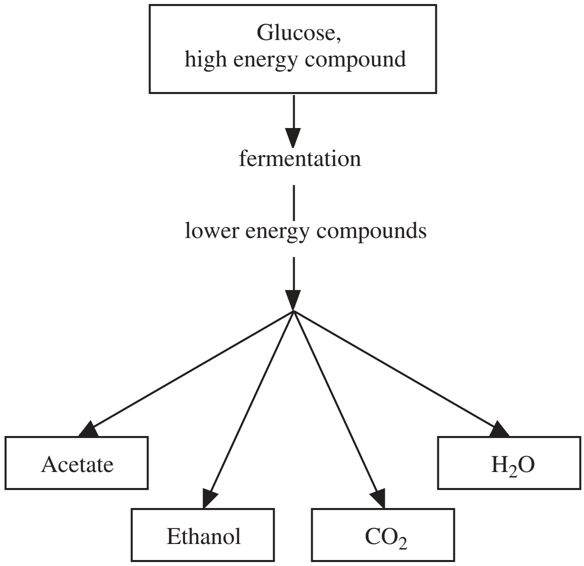


Figure 4.1 *The degradation of organic compounds results in the production of small compounds that contain less energy than the degraded compound. Inorganic compounds as well as organic compounds are produced from the degradation of organic compounds.*

of bicarbonate alkalinity (HCO_3^-) as its carbon source. *Nitrobacter winogradski* is found in activated sludge and trickling filter processes.

When substrate is degraded in a bacterial cell, energy is obtained from the electrons that are released from the broken chemical bonds of the substrate (Figure 4.2). The electrons released from the substrate are transferred along a series of electron carrier molecules—an electron transport system (Figure 4.3). As the electrons are transferred from one carrier molecule to another, some of the energy from the electrons is taken up by the carrier molecules to form high-energy phosphate bonds in the molecule adenosine triphosphate, or ATP (Figure 4.4). Phosphate bonds are the energy "currency" of the cell. When the cell needs energy, energy is "withdrawn" by breaking a phosphate bond. When this occurs, ATP is converted to adenosine diphosphate, or ADP. When the cell stores energy, energy is "deposited" by producing a phosphate bond. When this occurs, ADP is converted to ATP. Energy storage and release are based on a coupling and uncoupling of phosphate groups (PO_3^{2-}).

Eventually, the electrons are removed from the cell by a final electron carrier molecule. This molecule takes the electrons from the electron transport system and releases the electrons to the surrounding environment (Figure 4.5). Several final electron carrier molecules may be used by bacteria (Table 4.1). The molecule used by bacteria determines the form of respiration (Table 4.2).

The final electron carrier molecule used by the bacteria is dependent on several factors. These factors include 1) the presence or absence of the molecule, 2) the presence or absence of the necessary bacterial enzymes to use the molecule, and 3) the oxidation-reduction potential (ORP) of the wastewater or sludge that harbors the molecule and the bacteria (Table 4.3).

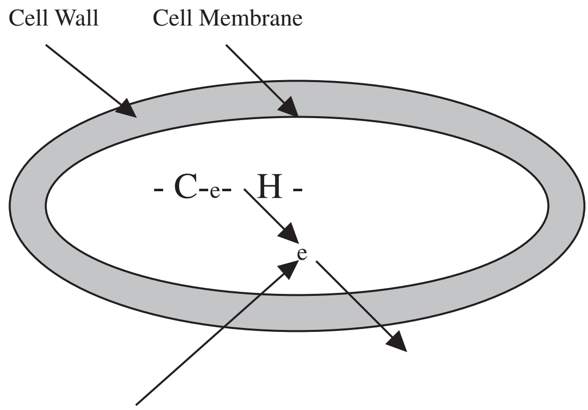


Figure 4.2 *Energy from degraded organic compounds is obtained by the capture of released electrons from broken chemical bonds. The captured electrons are transported along an electron transport system. The electrons release energy as they move along the transport system.*

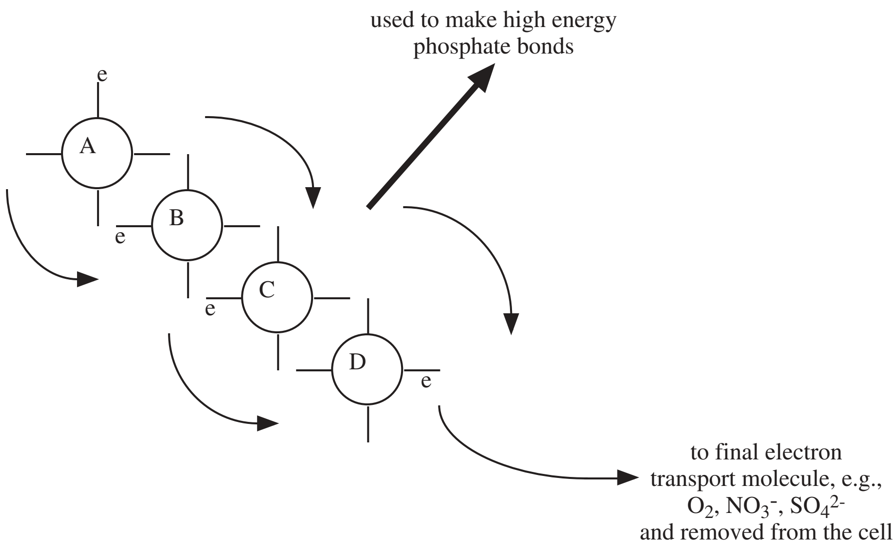


Figure 4.3 *The electron transport system consists of a series of interlocking, electron transport molecules that pass the electrons from one molecule to another. As the electrons are passed along the transport system, energy from the electrons is released and captured by the bacterial cell. The capture energy is used to form high energy phosphate bonds.*

For a final electron carrier molecule to be used by a bacterium, the molecule must be available and the bacterium must have the ability (enzymes) to use the molecule. Finally, the ORP of the bacterial environment (wastewater or sludge) determines the order or sequence of utilization of the final electron carrier molecules.

Respiration may be complete or incomplete. Complete respiration results in the transfer of the carbon in the organic substrate to carbon dioxide and new bacterial

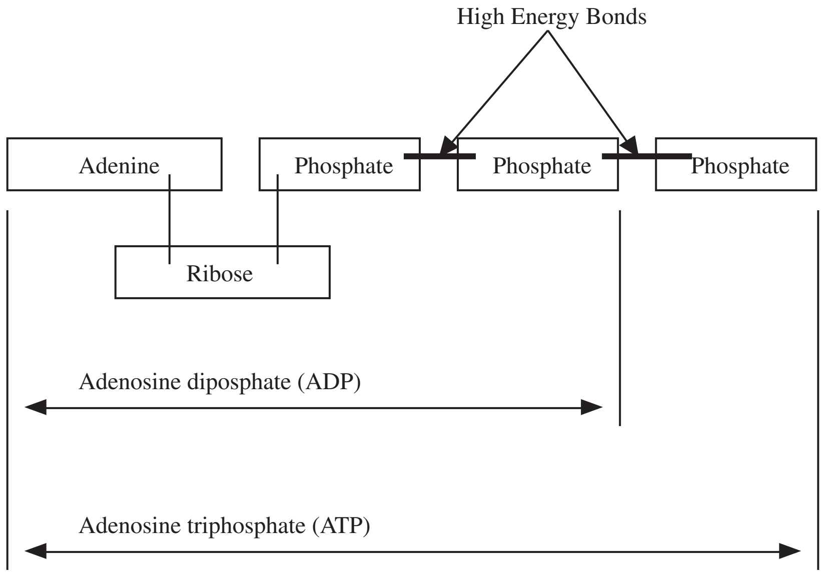


Figure 4.4 *Energy captured by bacterial cells by their electron transport system is used to form high energy phosphate bonds. When bonds are formed, ATP is produced. When the bonds are broken, energy is released and ADP is produced.*

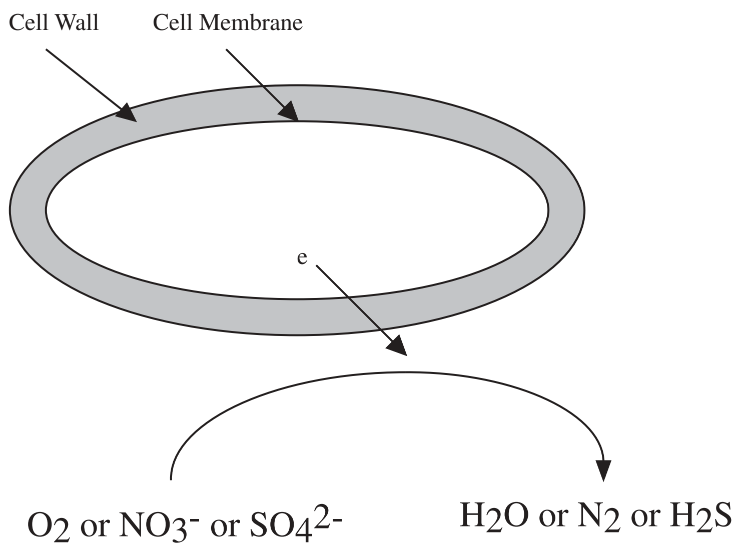


Figure 4.5 *Electrons released from the degradation of organic wastes are removed from the bacterial cell by a final, electron transport molecule such as free molecular oxygen, nitrate ion, and sulfate ions. The choice of final, electron transport molecule determines the form of respiration.*

TABLE 4.1 Final Electron Carrier Molecules in Order of Sequence of Utilization at Wastewater Treatment Plants

Order of Sequence of Utilization	Electron Carrier	Occurrence (Example)	Reduced Product
1	O_2	Aeration tank	H_2O
2	NO_3^-	Denitrification tank and secondary clarifier	N_2, N_2O
3	SO_4^{2-}	Secondary clarifier and thickener	S^{2-} (H_2S)
4	CH_2O*	Thickener and anaerobic digester	Volatile organic acids
5	CO_2	Anaerobic digester and sewer system	CH_4

* Organic compound

TABLE 4.2 Forms of Respiration

Respiration	Biochemical Reaction	Complete/Incomplete Respiration
Aerobic or oxic	$CH_2O + O_2 \rightarrow CO_2 + H_2O +$ cells	Complete
Anaerobic: anoxic (denitrification)	$CH_2O + NO_3^- \rightarrow CO_2 + H_2O + N_2 + N_2O +$ cells	Complete
Anaerobic: fermentation (sulfate reduction)	$CH_2O + SO_4^{2-} \rightarrow CO_2 + H_2O + H_2S +$ acids + alcohols + cells	Incomplete with exceptions
Anaerobic: fermentation (mixed acids and alcohol)	$CH_2O \rightarrow CO_2 + H_2O +$ acids + alcohols + cells	Incomplete
Anaerobic: fermentation (methane production)	$CH_2O + CO_2 \rightarrow H_2O + CH_4 +$ cells	Incomplete

TABLE 4.3 Oxidation-Reduction Potential and Respiration

Approximate Millivolts (mV)	Final Electron Carrier Molecule	Respiration Occurring
>+50	O_2	Aerobic or oxic
+50 to –50	NO_3^-	Anaerobic or anoxic
<–50	SO_4^{2-}	Anaerobic or sulfate reduction
<–100	CH_2O Organic molecule	Anaerobic or mixed acids and alcohol fermentation
<–300	CO_2 (carbonate, CO_3^{2-})*	Anaerobic or methane fermentation

* Carbon dioxide as carbonate

cells. Incomplete respiration results in the transfer of the carbon in the organic substrate to carbon dioxide, new bacterial cells, and organic products such as simple acids and alcohols.

The sequence of utilization for the carrier molecules is: O_2, NO_3^-, SO_4^{2-}, CH_2O, and CO_2. By using O_2 to degrade the organic compounds, bacterial cells obtain more energy from the organic compounds than through the use of any other carrier molecule (Table 4.4). With more energy, more bacterial growth (reproduction) or sludge is produced (Table 4.4). If O_2 is not available for bacterial use and NO_3^- is available,

TABLE 4.4 Final Electron Carrier Molecule, Energy Yield, and Cell (Sludge) Production

Final Electron Carrier Molecule	Form of Respiration	Energy Yield Rank	Pound of Cells Produced per Pound of COD Degraded
O_2	Aerobic or oxic	1	~0.4–0.6
NO_3^-	Anaerobic or anoxic	2	~0.4
SO_4^{2-}	Anaerobic: sulfate reduction	3	0.04–0.1
Organic molecule	Anaerobic: mixed acids and alcohol	4	0.04–0.1
CO_2	Anaerobic: methane production	5	0.02–0.04

NO_3^- is used next, if the bacteria have the enzymatic ability to use nitrate ions. The use of NO_3^- provides the second-largest energy yield for bacterial cells and the second-largest yield in bacterial growth (sludge production). Because of decreasing yields in energy and bacterial growth with different carrier molecules, there is a sequential order with respect to the choice of final electron carrier molecules. This order is determined by the ORP of the bacterial environment.

ORP is an indicator of the capacity of the molecules in the wastewater or sludge to release or gain electrons (oxidation or reduction, respectively). This measurement also is an indicator of the form of respiration that may occur (Table 4.3).

Generally, at values greater than +50 mV aerobic respiration may occur and from +50 to –50 mV anoxic respiration (denitrification) may occur. At values less than –100 mV, anaerobic respiration may occur. At values less than –50 mV sulfate (SO_4^{2-}) reduction (also known as fermentation) may occur. At values less than –100 mV, mixed acids and alcohol fermentation may occur. Methane fermentation may start at values less than –200 mV. However, in a mixed culture of fermenting organisms as would exist in an anaerobic digester, methane fermentation or the growth of methane-forming bacteria does not occur until the ORP is less than –300 mV. This is due to the inability of the methane-forming bacteria to successfully compete with other fermenting organisms at values greater than –300 mV.

The use of O_2 (Equation 4.1) and NO_3^- (Equation 4.2) as final electron carrier molecules results in complete degradation of CH_2O. In complete degradation, all of the carbon in the CH_2O is assimilated into new bacterial cells and CO_2. However, the use of NO_3^- results in a smaller production of bacterial cells and a greater production of CO_2 (Table 4.4).

$$CH_2O + O_2 \rightarrow \text{cells} + CO_2 + H_2O \tag{4.1}$$

$$CH_2O + NO_3^- \rightarrow \text{cells} + CO_2 + H_2O + N_2 + N_2O \tag{4.2}$$

The use of nitrate ions by bacteria to degrade carbonaceous compounds is known as anoxic respiration or denitrification. The occurrence of denitrification in secondary clarifiers of activated sludge processes is known as rising sludge or clumping. Many different groups of bacteria are capable of using nitrate ions to

TABLE 4.5 Significant Organic Compounds Produced During Anaerobic Fermentation

Name	Formula
Acetate	CH_3COOH
Acetone	CH_3COCH_3
Acetaldehyde	CH_3CHO
Butanol	$CH_3(CH_2)_2CH_2OH$
Butanone	$C_2H_5COCH_3$
Butyraldehyde	C_2H_5CHO
Caproic acid	$CH_3(CH_2)_4COOH$
Formaldehyde	CH_2O
Formate	$HCOOH$
Ethanol	CH_3CH_2OH
Lactate	$CH_3CHOHCOOH$
Methane	CH_4
Methanol	CH_3OH
Propanol	$CH_3CH_2CH_2OH$
Propionate	CH_3CH_2COOH
Valeric acid	$CH_3(CH_2)_3COOH$

TABLE 4.6 Groups of Chemolithotrophs Found in Wastewater Treatment Plants

Group	Substrate	Product
Ammonium oxidizers	NH_4^+	NO_2^-
Hydrogen bacteria	H_2	H^+
Iron bacteria	Fe^{2+}	Fe^{3+}
Nitrite oxidizers	NO_2^-	NO_3^-
Sulfur bacteria	H_2S	S^o
	S^o	SO_3^{2-}
	SO_3^{2-}	SO_4^{2-}

degrade carbonaceous compounds. These bacteria include facultative and anaerobic bacteria.

With exceptions, all other forms of respiration (anaerobic fermentation) are incomplete. During these forms of respiration, the carbon within the CH_2O is assimilated into new bacterial cells, CO_2, and a variety of simplistic, soluble organic molecules, mostly acids and alcohols (Table 4.5). Because some of the carbon from the CH_2O is assimilated into a variety of organic molecules, the production of bacterial cells is greatly reduced (Table 4.4). However, several sulfate-reducing bacteria are capable of complete respiration. These sulfate-reducing bacteria provide the exceptions for complete respiration under anaerobic respiration.

For most obligate anaerobic bacteria to grow, the absence of free molecular oxygen and a low redox potential are required. Methane-forming bacteria only grow in anaerobic digester sludge with a redox potential less than –300 mV. Also, the digester sludge must have thiol group-containing (–SH) compounds. These compounds produce a reducing environment.

Sulfates, carbonates (CO_3^{2-}), and bicarbonates are the primary electron carrier molecules for facultative anaerobic and anaerobic bacteria. If sulfate is used as the final electron carrier molecule, dissimilatory sulfate reduction occurs (Equation 4.2). During dissimilatory sulfate reduction, sulfate serves as the electron acceptor and hydrogen sulfide (H_2S) is produced. Only a relatively small number of genera of bacteria are capable of dissimilatory sulfate reduction. *Desulfovibrio* is the predominant genus responsible for the conversion of sulfate to hydrogen sulfide. *Desulfotomaculum* also is capable of reducing sulfate. Conversely, in an oxidizing environment, sulfides (HS^-) are oxidized to sulfate. Genera of bacteria containing species of sulfide-oxidizing bacteria are *Thiobacillus*, *Thiobacterium*, and *Thiospira*.

$$SO_4^{2-} + CH_2O \rightarrow H_2S + CO_2 + H_2O \tag{4.2}$$

In the absence of an inorganic final electron carrier molecule, an organic compound may be used to achieve respiration. If an organic compound is used, mixed-acid fermentation occurs.

The substrate degraded or electron-releasing compound used during respiration may be organic, for example, glucose, or inorganic, for example, ammonium ions (NH_4^+). Bacteria that respire by using organic substrates are organotrophs, whereas bacteria that respire by using inorganic substrates are chemolithotrophs. Several important groups of chemolithotrophs are found in wastewater treatment processes (Table 4.6). These groups include ammonium oxidizers, hydrogen bacteria, iron bacteria, nitrite oxidizers, and sulfur bacteria.

5

Anaerobic Food Chain

In natural habitats that are void of free molecular oxygen and nitrate ions, insoluble and complex organic compounds are degraded by different groups of bacteria through a variety of anaerobic or fermentative biochemical reactions. These reactions result in the production of soluble and simplistic organic compounds. These compounds do not accumulate in natural habitats.

As one group of bacteria produces soluble compounds they are quickly degraded as substrate by another group of bacteria. The bacteria form a chain—an anaerobic food chain—in which large, complex compounds are degraded to more simplistic compounds as they are passed along the food chain (Figure 5.1).

In freshwater habitats, methane fermentation is the terminal link in the anaerobic food chain. Here, complex organic compounds have been degraded or reduced to methane, carbon dioxide, and minerals. Some of the carbon dioxide produced during the degradation of organic compounds is reduced to form methane.

For organic compounds to be degraded through the food chain, the compounds must be degraded to simplistic organic and inorganic compounds that can be used as substrate by methane-forming bacteria. These compounds include the organics formate, methanol, methylamine, and acetate and the inorganics hydrogen and carbon dioxide.

Methane is produced by methane-forming bacteria from organic compounds such as acetate (Equation 5.1) or from the combination of the inorganics carbon dioxide [as bicarbonate (HCO_3^-) or carbonate (CO_3^{2-})] with hydrogen (H_2) (Equations 5.2 and 5.3).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (5.1)$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad (5.2)$$

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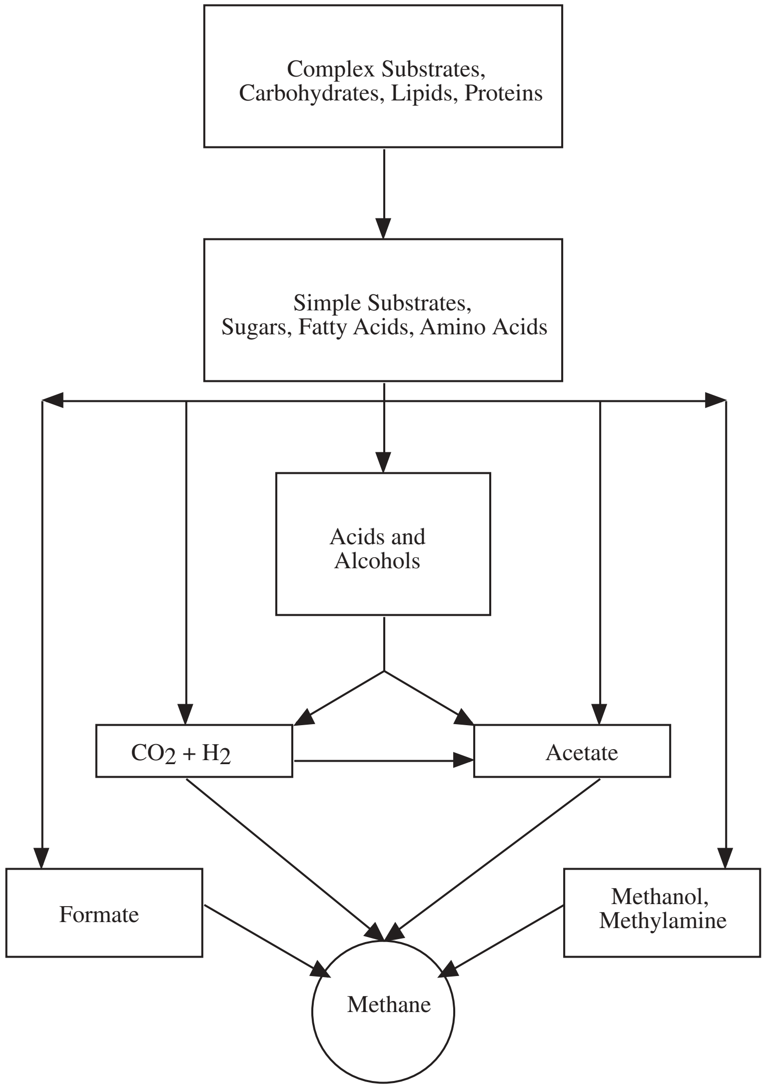


Figure 5.1 *The anaerobic food chain consists of several groups of facultative anaerobes and anaerobes that degrade and transform complex organic compounds into simplistic organic compounds. The final organic compound produced in the anaerobic food is methane. This compound is the most reduced form of carbon.*

$$4H_2 + CO_3^{2-} + 2H^+ \rightarrow CH_4 + 3H_2O \quad (5.3)$$

Methane is the most reduced organic compound. The production of methane is the final step of the anaerobic food chain. Methane-forming bacteria are responsible for this step, and therefore they are of critical importance for the success of the anaerobic food chain.

Organic compounds that cannot be used directly as substrate by methane-forming bacteria can be used indirectly if they are converted to compounds such as

acetate. Examples of compounds that can be converted to acetate include butyrate and propionate.

Within the anaerobic food chain there are syntrophic relationships between bacteria. In these relationships at least two different bacteria are involved and the activity of one organism is dependent on the activity of another organism. An example of a syntrophic relationship in the anaerobic food chain is the association between hydrogen-producing bacteria and hydrogen-consuming bacteria. In this association hydrogen-producing bacteria degrade organic compounds to more simplistic compounds and hydrogen (Equation 5.4).

$$\text{glucose} + 4H_2O \rightarrow 2\ \text{acetate} + 2HCO_3^- + 2H^+ + 4H_2 \quad (5.4)$$

However, the degradation of organic compounds by hydrogen-producing bacteria occurs only if the partial pressure of hydrogen is kept low, that is, $<10^{-4}$ atmospheres. Therefore, it is essential that hydrogen does not accumulate to a partial pressure $\geq 10^{-4}$ atmospheres. In the anaerobic food chain, hydrogen is consumed and hydrogen partial pressure is maintained at a low value by hydrogen-consuming bacteria, including methane-forming bacteria. These organisms combine hydrogen with carbon dioxide to produce methane.

As long as the hydrogen partial pressure is maintained at a low level, hydrogen-producing bacteria continue to degrade organic compounds and the anaerobic food chain continues to function. Fermentation under low partial pressure of hydrogen helps to ensure that fermentation products other than methane and carbon dioxide do not accumulate.

The partial pressure of hydrogen in the rumen, in mud, and in anaerobic digesters is kept low by the microbial activity of methane-forming bacteria. This favors the organisms that produce hydrogen and acetate. The maintenance of a low hydrogen pressure is necessary for proper microbial activity within the anaerobic food chain.

Acetate is the most important organic compound in the anaerobic food chain. Acetate is the substrate most commonly used by methane-forming bacteria and may be degraded in the absence of sulfate. In the presence of sulfate, acetate is not split to methane and carbon dioxide.

6

Fermentation

The term "fermentation" was first used by Pasteur to define respiration in the absence of free molecular oxygen. Fermentation can be broadly defined as respiration that occurs in the dark (no photosynthesis) and does not involve the use of free molecular oxygen, nitrate ions, or nitrite ions as the final electron acceptors of degraded organic compounds. Therefore, respiration may occur through several fermentative pathways including sulfate reduction, mixed acid production, and methane production.

Fermentation is a form of anaerobic respiration. The bacteria that perform fermentation are facultative anaerobes and anaerobes. Fermentation involves the transformation of organic compounds to various inorganic and organic products. During fermentation a portion of an organic compound may be oxidized while another portion is reduced. It is from this oxidation-reduction of organic compounds that fermenting bacteria obtain their energy and produce numerous simplistic and soluble organic compounds.

Fermentative bacteria are capable of performing a variety of oxidation-reduction reactions involving organic compounds, carbon dioxide, carbon monoxide (CO), molecular hydrogen, and sulfur compounds. Fermentative bacteria include facultative anaerobes, aerotolerant anaerobes, and strict anaerobes. Some fermentative bacteria such as the clostridia (Table 6.1) and *Escherichia coli* (Table 6.2) produce a large variety of products, whereas other fermentative bacteria such as *Acetobacterium* produce a very small number of products. As environmental or operational conditions change, for example, pH and temperature, the bacteria that are active and inactive also change. These changes in activity are responsible for changes in the types and quantities of compounds that are produced through fermentation.

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TABLE 6.1 Fermentative Products of Clostridia

Organic	Inorganic
Acetate	Carbon dioxide
Acetone	Hydrogen
Butanol	
Butyrate	
Ethanol	
Lactate	

TABLE 6.2 Fermentative Products of *Escherichia coli*

Organic	Inorganic
Acetate	Carbon dioxide
2,3-Butanediol	Hydrogen
Ethanol	
Formate	
Lactate	
Succinate	

Some products of fermentative bacteria such as acetate and formate can be used as substrate for methane-forming bacteria. Some products of fermentative bacteria such as butyrate and propionate may be used as substrate for methane-forming bacteria only if they are converted to compounds such as acetate and formate. Some products of fermentative bacteria cannot be used as substrate by methane-forming bacteria. Therefore, changes in operational conditions of an anaerobic digester such as pH and temperature determine which fermentative bacteria are dominant and consequently which fermentative products are dominant. These products in turn significant influence the activity of methane-forming bacteria and the efficiency of the anaerobic digester process.

A relatively large variety of organic compounds and inorganic compounds are produced through fermentation. The compounds obtained through fermentation are dependent on the compounds fermented, the bacteria involved in the fermentation process, and the operational conditions that exist during fermentation. There are several types of fermentation, which are classified according to the major end products obtained in the fermentation process (Equation 6.1). The types of fermentation include acetate, alcohol (ethanol), butyrate, lactate, mixed acid, mixed acid and butanediol, propionate and succinate, sulfide, and methane (Figure 6.1).

$$\text{reactants} \rightarrow \text{products} \tag{6.1}$$

ACETATE FERMENTATION

Acetate is produced in several fermentative pathways. A large diversity of bacteria, collectively known as acetogenic or acetate-forming bacteria, produces

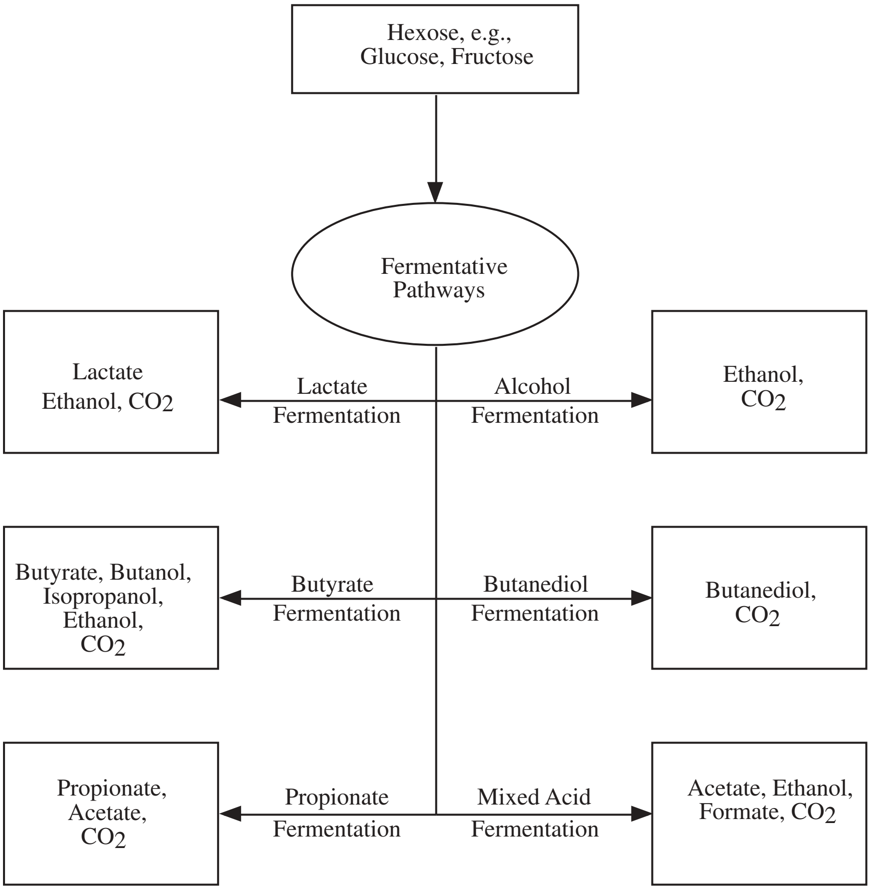


Figure 6.1 *There are numerous types of fermentation. The type of fermentation that occurs is classified or named after the major product(s) obtained in the fermentation process.*

nongaseous acetate. These organisms include bacteria in the genera *Acetobacterium*, *Clostridium*, and *Sporomusa*. Some acetogenic bacteria are thermophilic.

Several biochemical reactions are used by acetogenic bacteria to produce acetate. Most acetogenic bacteria produce acetate from H_2 and CO_2 (Equation 6.2), while some produce acetate from H_2O and carbon monoxide (Equation 6.3). Some acetogenic bacteria produce acetate from CO_2 and methanol (Equation 6.4), and often six-carbon sugars or hexoses are degraded to acetate (Equation 6.5). Even propionate is converted to acetate.

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \tag{6.2}$$

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \tag{6.3}$$

$$4CH_3OH + CO_2 \rightarrow 3CH_3COOH + 2H_2O \tag{6.4}$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \tag{6.5}$$

ALCOHOL (ETHANOL) FERMENTATION

Although alcohol fermentation is the domain of yeast (mostly *Saccharomyces*), alcohol is produced by several species of bacteria in the genera *Erwinia*, *Sarcina*, and *Zymomonas*. These organisms produce ethanol from the anaerobic degradation of hexoses such as glucose (Equation 6.6). At relatively low pH values (<4.5), alcohol is produced by bacteria in the genera *Enterobacter* and *Serratia*.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (6.6)$$

BUTYRATE FERMENTATION

Butyrate ($CH_3CH_2CH_2COOH$) is a major fermentative product of many bacteria. Strict anaerobes in the genera *Clostridium* and *Butyrivibrio* ferment a variety of sugars to produce butyrate (Equation 6.7). Under low pH values (<4.5), several clostridia produce small amounts of acetone and *n*-butanol. *n*-Butanol is highly toxic to bacteria because of its interference with cellular membrane functions.

$$\text{hexose} \rightarrow CH_3CH_2CH_2COOH \quad (6.7)$$

LACTATE FERMENTATION

A common product of many fermentative reactions is lactate. The production of lactate is achieved by the aerotolerant, strictly fermentative lactate-forming bacteria (Table 6.3). Lactate-forming bacteria are highly saccharolytic.

There are three biochemical reactions for lactate production from sugars such as glucose (Equations 6.8, 6.9, and 6.10). In addition to glucose, other sugars fermented by lactate-forming bacteria include fructose, galactose, mannose, saccharose, lactose, maltose, and pentoses.

$$\text{glucose} \rightarrow 2 \text{ lactate} \quad (6.8)$$

$$\text{glucose} \rightarrow \text{lactate} + \text{ethanol} + CO_2 \quad (6.9)$$

$$2 \text{ glucose} \rightarrow 2 \text{ lactate} + 3 \text{ acetate} \quad (6.10)$$

TABLE 6.3 Major Genera of Lactate-forming Bacteria

Bifidobacterium
Lactobacillus
Leuconostoc
Pediococcus
Sporolactobacillus
Streptococcus

TABLE 6.4 Major Genera of Propionate-forming Bacteria and Succinate-forming Bacteria

Bacteroides
Clostridium
Peptostreptococcus
Ruminococcus
Selenomonas
Succinivibrio
Veillonella

PROPIONATE AND SUCCINATE FERMENTATION

Anaerobic propionibacteria or propionate-forming bacteria (Table 6.4) ferment glucose and lactate (Equations 6.11 and 6.12). Lactate, the major end product of lactate fermentation, is the preferred substrate of propionate-forming bacteria. Although succinate ($HOOCCH_2CH_2COOH$) usually is an intermediate product of fermentation, some succinate is produced as an end product.

$$1.5 \text{ glucose} \rightarrow 2 \text{ propionate} + \text{acetate} + CO_2 \tag{6.11}$$

$$3 \text{ lactate} \rightarrow 2 \text{ propionate} + \text{acetate} + CO_2 \tag{6.12}$$

Propionate is a major substrate of acid fermentation that can be converted to acetate and then used in methane production. Propionate increases to relatively high concentrations under adverse operational conditions.

SULFIDE FERMENTATION

Sulfate is reduced to sulfide by bacteria for two purposes. First, bacteria use sulfate as the principal sulfur nutrient. This is done by enzyme systems that reduce sulfate to sulfide. The reduction of sulfate to sulfide and its incorporation as a nutrient into cellular material is termed assimilatory sulfate reduction. Second, during sulfide fermentation or desulfurication, sulfate is reduced to sulfide as organic compounds are oxidized. Because the sulfide produced through fermentation is released to the environment and not incorporated into cellular material, sulfide fermentation is also known as dissimilatory sulfate reduction.

There are two groups of sulfate-reducing bacteria—incomplete oxidizers and complete oxidizers (Table 6.5). Incomplete oxidizers degrade organic compounds to new bacterial cells, carbon dioxide, and acetate, ethanol, formate, lactate, and propionate, whereas complete oxidizers degrade organic compounds to new bacterial cells and carbon dioxide.

METHANE FERMENTATION

Three types of methane-forming bacteria achieve methane production—two groups of obligate chemolithotrophic methanogens and one group of methylotrophic

TABLE 6.5 Genera of Sulfate-reducing Bacteria

Genus	Species of Incomplete Oxidizers	Species of Complete Oxidizers
Desulfobacter		X
Desulfobulbus	X	
Desulfococcus		X
Desulfonema		X
Desulfosarcina		X
Desulfotomaculum	X	X
Desulfovibrio	X	

methanogens. Chemolithotrophic methanogens produce methane from carbon dioxide and hydrogen (Equation 6.13) or formate (Equation 6.14). Carbon monoxide also may be used by some chemolithotrophic methanogens in the production of methane (Equation 6.15). Methylotrophic methanogens produce methane by using methyl group ($-CH_3$)-containing substrates such as methanol (Equation 6.16), methylamine (Equation 6.17), and acetate (Equation 6.18). These organisms produce methane directly from the methyl group and not via carbon dioxide.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (6.13)$$

$$2HCOOH \rightarrow CH_4 + CO_2 \quad (6.14)$$

$$4CO + H_2O \rightarrow CH_4 + 3CO_2 \quad (6.15)$$

$$3CH_3OH + 3H_2 \rightarrow 3CH_4 + 3H_2O \quad (6.16)$$

$$4\ (CH_3)_3\text{–}N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \quad (6.17)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (6.18)$$

MIXED-ACID FERMENTATION AND MIXED-ACID AND BUTANEDIOL FERMENTATION

A large variety of bacteria in the genera *Enterobacter*, *Escherichia*, *Erwinia*, *Salmonella*, *Serratia*, and *Shigella* are responsible for mixed acid fermentation. These organisms ferment sugars to a mixture of acids—acetate, formate, lactate, and succinate. Carbon dioxide, hydrogen, and ethanol also are produced. The prevalence of acids among the products of mixed-acid fermentation account for the name of the fermentation process.

Bacteria in the genera *Enterobacter* and *Erwinia* also produce 2,3-butanediol in addition to acids. Production of butanediol increases with decreasing pH (<6).

In anaerobic digesters, acid production (Equation 6.19) takes place simultaneously with methane production (Equation 6.20). Although several acids are produced during acid fermentation, acetate is the primary substrate used for methane production in an anaerobic digester.

$$\text{polysaccharides} \rightarrow \text{glucose} — \textit{Escherichia} \rightarrow \text{acetate} \quad (6.19)$$

$$\text{acetate} — \textit{Methanococcus} \rightarrow \text{methane} + \text{carbon dioxide} \quad (6.20)$$

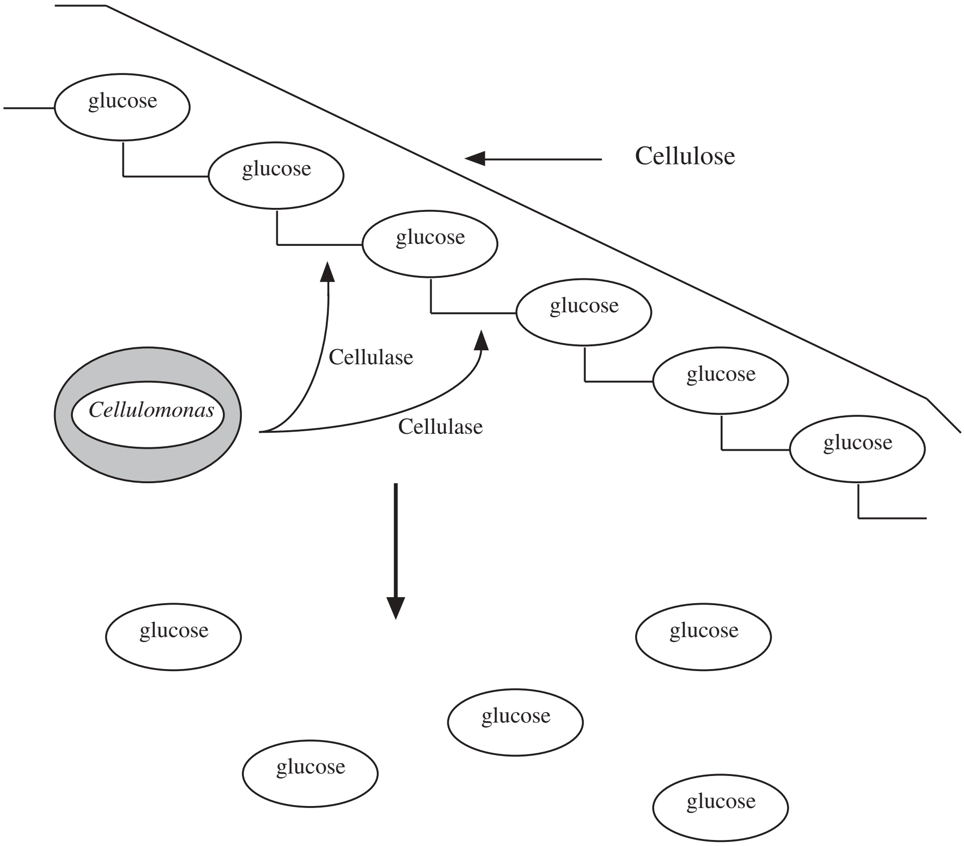


Figure 6.2 *Cellulose is an insoluble starch or particulate organic waste. Cellulose must be hydrolyzed before it can be degraded. Exoenzymes releases by specific hydrolytic bacteria such as* Cellulomonas *add water to the chemical bonds between the glucose units that make up cellulose. Once the chemical bonds are hydrolyzed, glucose goes into solution and is absorbed by numerous bacteria and degraded inside the bacterial cells.*

For methane production to occur in municipal anaerobic digesters, complex, insoluble organic compounds must be converted to simplistic, soluble compounds that can enter bacterial cells. For example, cellulose is converted through bacterial action to numerous small and soluble molecules of glucose (Figure 6.2). Through acid fermentation, glucose is converted to acetate and, finally, acetate is converted to methane. The majority of large, complex, and insoluble organic compounds in municipal anaerobic digesters consist of three basic substrates—carbohydrates, lipids, and proteins.

The fermentation of organic compounds by acid-forming bacteria and methane-forming bacteria also results in the growth of new bacterial cells or sludge. However, the energy obtained by the bacteria during fermentation (anaerobic respiration) is relatively small (compared with aerobic respiration) and this small quantity of energy results in production of a relatively small quantity of cells or sludge (Table 6.6).

TABLE 6.6 Sludge Production or Yield (kg VSS/kg COD) for Volatile Acid-forming and Methane-forming Bacteria

Bacterial Group	Yield (kg VSS/kg COD)
Volatile acid-forming bacteria	0.15
Methane-forming bacteria	0.03

The production of acetate through fermentation is accompanied by the production of hydrogen. Acetate-forming bacteria produce acetate—the major substrate used by methane-forming bacteria—as long as the hydrogen partial pressure is low. A low partial hydrogen pressure is maintained in the digester as long as methane-forming bacteria use hydrogen to form methane. By removing hydrogen, acetate production is favored.

7

Anaerobic Digestion Stages

The anaerobic digestion process and production of methane is divided into stages. Three stages often are used to illustrate the sequence of microbial events that occur during the digestion process and the production of methane (Figure 7.1). These stages are hydrolysis, acid forming, and methanogenesis. The critical biochemical reactions within these stages are presented in Figure 7.2.

The anaerobic digestion process proceeds efficiently if the degradation rates of all three stages are equal. If the first stage is inhibited, then the substrates for the second and third stages will be limited and methane production decreases. If the third stage is inhibited, the acids produced in the second stage accumulate. The inhibition of the third stage occurs because of an increase in acids and, consequently, loss of alkalinity and decrease in pH. The most common upsets of anaerobic digesters occur because of inhibition of methane-forming bacteria—the third stage.

The anaerobic digestion process contains different groups of bacteria. These groups work in sequence, with the products of one group serving as the substrates of another group. Therefore, each group is linked to other groups in chainlike fashion, with the weakest links being acetate production and methane production.

STAGE 1—HYDROLYSIS STAGE

In the anaerobic digester complex insoluble compounds such as particulate and colloidal wastes undergo hydrolysis. Particulate and colloidal wastes consist of carbohydrates, fats, and proteins. These wastes are polymeric substances, that is, large insoluble molecules consisting of many small molecules joined together by unique chemical bonds. The small molecules are soluble and quickly go into solu-

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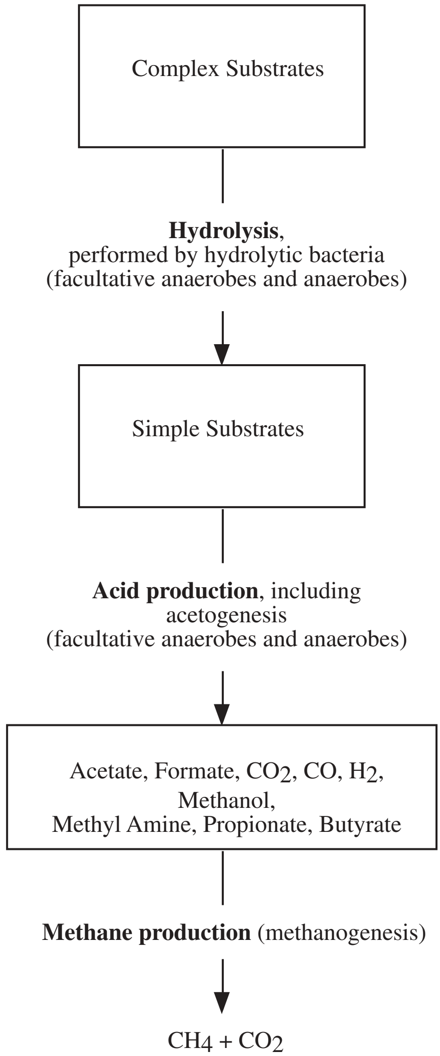


Figure 7.1 *There are three basic stages of the anaerobic digestion process and production of methane. These stages include the solublization of complex organic compounds or hydrolysis, the production of simplistic acids or acid production, and the formation of methane or methane production.*

tion once the chemical bonds are broken. Hydrolytic bacteria or facultative anaerobes and anaerobes that are capable of performing hydrolysis achieve breakage of these unique bonds. Hydrolysis is the splitting (lysis) of a compound with water (hydro). An example of an insoluble compound that undergoes hydrolysis in an anaerobic digester is cellulose (Figure 7.3).

Cellulose $[(C_6H_{12}O_6)_n]$ is an insoluble starch that is commonly found in primary and secondary municipal sludges. Cellulose may make up approximately 15% of the dry weight of the sludges. Cellulose consists of many sugar units or mers of glucose $(C_6H_{12}O_6)$ joined together by unique chemical bonds. Although glucose is soluble

Hydrolysis

Complex carbohydrates -----> Simple sugars
Complex lipids -----> Fatty acids
Complex proteins -----> Amino acids

Acid Production

Simple sugars + fatty acids + amino acids -----> organic acids, including acetate + alcohols

Acetogenesis (acetate production)

Organic acids + alcohols -----> acetate

Methane production: acetoclastic methanogenesis

Acetate → $CH_4 + CO_2$

Methane production: hydogenotrophic methanogenesis

$H_2 + CO_2$ → CH_4

Methane production: methyltrophic methanogenesis

Methanol → $CH_4 + H_2O$

Figure 7.2 *The critical biochemical reactions in the anaerobic digestion process and production of methane include hydrolysis, acid production, acetogenesis, and methane production. Methane production may occur through the use of acetate, hydrogen and carbon dioxide, and methanol.*

in water, the joining of the many mers of glucose by unique chemical bonds results in the production of the insoluble polymer cellulose.

When cellulose is hydrolyzed in an anaerobic digester, many molecules of soluble glucose are released (Equation 7.1). Cellulose is hydrolyzed by the hydrolytic bacterium *Cellulomonas*. The bacterium is able to hydrolyze cellulose because it processes the enzyme cellulase, which is capable of breaking the bonds between the mers of glucose.

$$(C_6H_{12}O_6)_n + H_2O \rightarrow nC_6H_{12}O_6 \qquad (7.1)$$

Anaerobic digesters at industrial wastewater treatment plants that degrade simplistic, soluble organic compounds such as glucose do not experience hydrolysis or stage 1. However, complex, soluble organic compounds such as table sugar (sucrose) must be hydrolyzed. Table sugar is a disaccharide consisting of two 6-carbon sugars, glucose and fructose, that are bonded together. Although soluble in water, table sugar is too complex to enter a bacterial cell where it can be degraded. Table sugar

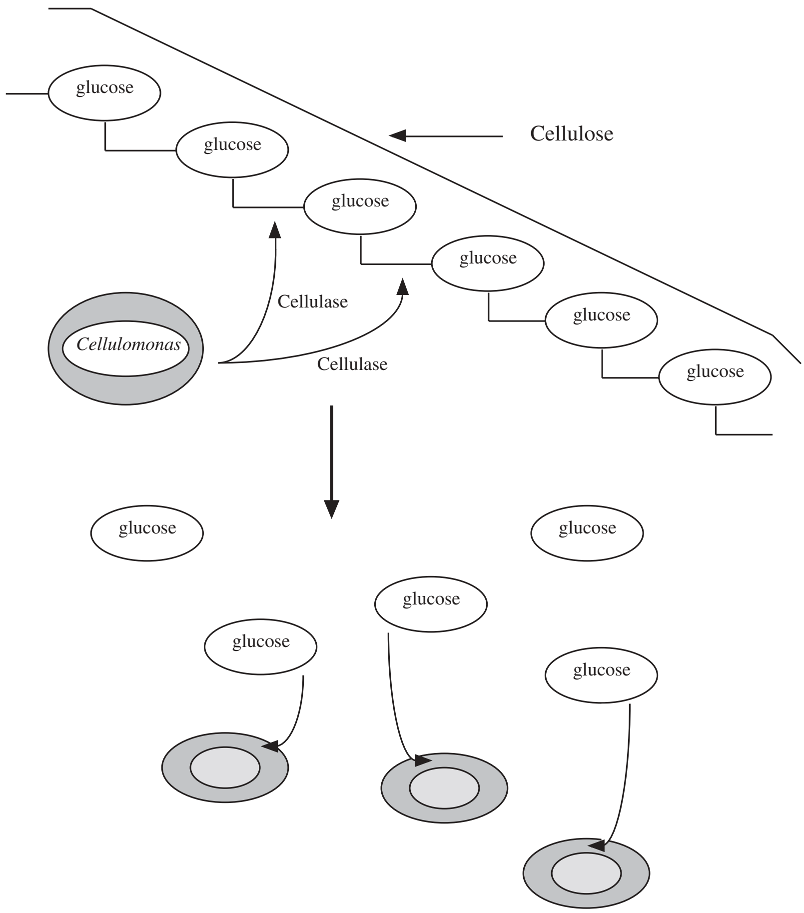


Figure 7.3 *Cellulose is an insoluble starch or particulate organic waste. Cellulose must be hydrolyzed before it can be degraded. Exoenzymes releases by specific hydrolytic bacteria such as* Cellulomonas *add water to the chemical bonds between the glucose units that make up cellulose. Once the chemical bonds are hydrolyzed, glucose goes into solution and is absorbed by numerous bacteria and degraded inside the bacterial cells.*

must be hydrolyzed to glucose and fructose (Equation 7.2). After hydrolysis glucose and fructose can enter a bacterial cell and be degraded (Figure 7.4).

$$\text{sucrose} + H_2O \rightarrow \text{glucose} + \text{fructose} \qquad (7.2)$$

STAGE 2—ACID-FORMING STAGE

In the acid-forming stage, soluble compounds produced through hydrolysis or discharged to the digester are degraded by a large diversity of facultative anaerobes and anaerobes through many fermentative processes. The degradation of these compounds results in the production of carbon dioxide, hydrogen gas, alcohols, organic

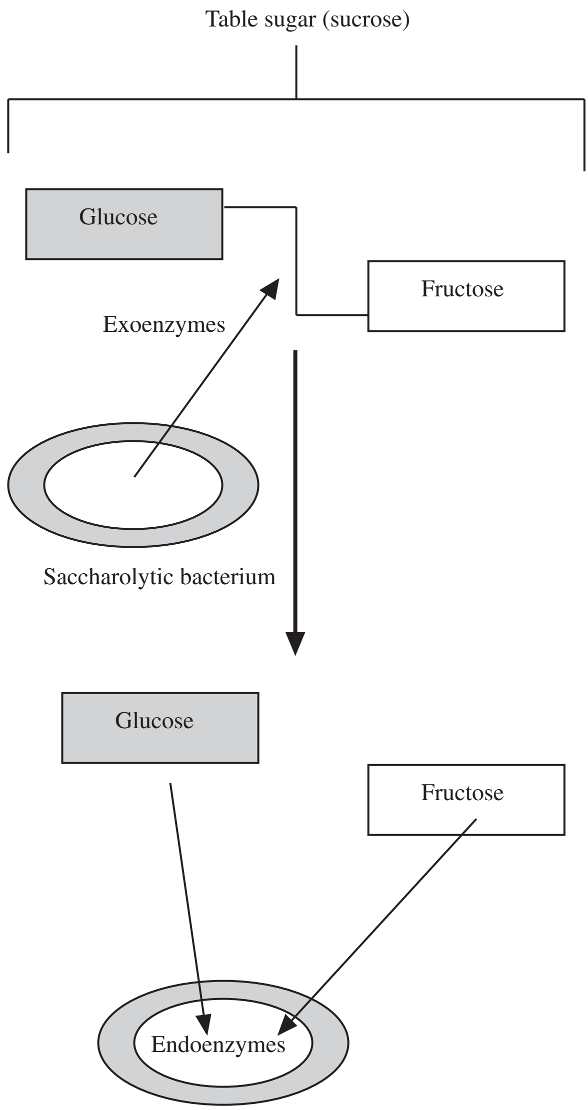


Figure 7.4 *Although table sugar is soluble in water, table sugar is too large and complex to enter a bacterial cell. In order for bacteria to degrade table sugar, the sugar must be hydrolyzed to its individual units, glucose and fructose. Once hydrolyzed, glucose and fructose can enter the bacterial cell and be degraded. Hydrolysis of table sugar is achieved through exoenzymes, while degradation is achieved through endoenzymes.*

acids, some organic-nitrogen compounds, and some organic-sulfur compounds. (Table 7.1). The most important of the acids is acetate.

Acetate is the principal organic acid or volatile acid used as a substrate by methane-forming bacteria. Carbon dioxide and hydrogen can be converted directly to acetate or methane. The presence of organic-nitrogen compounds and organic-sulfur compounds is due to the degradation of amino acids and proteins. The conversion of large soluble organic compounds to small soluble organic compounds

TABLE 7.1 Major Acids and Alcohols Produced Through Fermentation Processes in Anaerobic Digesters

Name	Formula
Acetate	CH_3COOH
Butanol	$CH_3(CH_2)_2CH_2OH$
Butyrate	$CH_3(CH_2)_2CH_2COOH$
Caproic acid	$CH_3(CH_2)_4COOH$
Formate	$HCOOH$
Ethanol	CH_3CH_2OH
Lactate	$CH_3CHOHCOOH$
Methanol	CH_3OH
Propanol	$CH_3CH_2CH_2OH$
Propionate	CH_3CH_2COOH
Succinate	$HOOCCH_2CH_2COOH$

TABLE 7.2 Alcohols, Organic-nitrogen Compounds, and Organic Acids Used as Substrates by Methane-forming Bacteria

Substrate	Chemical Formula
Acetate	CH_3COOH
Formate	$HCOOH$
Methanol	CH_3OH
Methylamine	CH_3NH_2

TABLE 7.3 Alcohol and Organic Acids Used Indirectly as Substrates by Methane-forming Bacteria

Substrate	Chemical Formula
Ethanol	CH_3CH_2OH
Butyrate	$CH_3CH_2CH_2COOH$
Propionate	CH_3CH_2COOH

results in little change in the organic strength of the compounds. Some of the organic compounds are converted to organic acids and alcohols, and some are converted to new bacterial cells. It is only in methane formation or the methanogenic stage that degradable organics are removed as methane and carbon dioxide.

Within the pool of organic acids, alcohols, and organic-nitrogen compounds, there are those that can be used directly as a substrate by methane-forming bacteria (Table 7.2) and those that can be used indirectly (Table 7.3) if they are degraded to acetate by fermentative bacteria. If the methane-forming bacteria do not degrade the products of the second stage, the products will accumulate and produce an acid medium.

Acetate can be produced not only through the fermentation of soluble organic compounds but also through acetogenesis. Acetogenesis occurs in the acid-forming stage. Here, many of the acids and alcohols, for example, butyrate, propionate, and ethanol, produced during the acid-forming stage may be degraded to acetate that

can be used as a substrate by methane-forming bacteria. The production of acetate is accomplished through the activity of acetogenic or acetate-forming bacteria.

STAGE 3—METHANOGENESIS STAGE

In the methanogenic stage, methane is formed mostly from acetate and carbon dioxide and hydrogen gas. Methane is also formed from some organic compounds other than acetate (Table 7.2). Therefore, all other fermentative products must be converted to compounds that can be used directly or indirectly by methane-forming bacteria. Acids, alcohols, and organic-nitrogen compounds that are not degraded by methane-forming bacteria accumulate in the digester supernatant. The accumulation of these compounds is responsible for the relatively high organic strength or carbonaceous biochemical oxygen demand (cBOD) of the supernatant.

As long as the "working velocity" of acid-producing bacteria and methane-forming bacteria are roughly the same, the metabolic activity of the methanogenic stage is safeguarded. If the methanogenic stage is safeguarded, the acids are broken down and a slightly alkaline medium is achieved from the overall process because of the formation of ammonia (NH_3) from amino groups ($-NH_2$) that are released through the degradation of proteins and amino acids.

Ammonia released in the sludge often reacts with carbon dioxide and water, resulting in the production of ammonium carbonate that provides alkalinity to the system (Equation 7.3). The ammonium carbonate is available to react with the volatile acids that are present in the sludge. This reaction results in the production of volatile acid salts (Equation 7.4).

$$NH_3 + CO_2 + H_2O \rightarrow NH_4HCO_3 \tag{7.3}$$

$$NH_4HCO_3 + RCOOH^* \rightarrow RCOONH_4 + H^+ + HCO_3^- \tag{7.4}$$

*R represents the non-carboxyl (–COOH) portion of the volatile acid.

The decomposition of complex organic compounds to methane proceeds as rapidly as the compounds can be converted to substrates that are capable of being used by methane-forming bacteria. Within the anaerobic conversions and degradations of organic compounds, the production of acetate is the rate-limiting step or "bottleneck" in the final degradation of complex organic compounds. For organic compounds that are poorly biodegradable, the hydrolysis stage may become the rate-limiting step.

Part II

Substrates, Products, and Biogas

8

Substrates and Products

In chemical reactions there are reactants and products (Equation 8.1). During chemical reactions, reactants (chemical compounds) undergo change and often release energy (heat) to the environment. The changes that occur to the reactants result in the formation of products (new chemical compounds). Often, a catalyst may be involved in a chemical reaction. The catalyst accelerates the rate of the chemical reaction and may be changed or consumed.

$$\text{reactants — catalyst} \rightarrow \text{products} \tag{8.1}$$

Chemical reactions that occur inside bacterial cells are known as biochemical reactions. In biochemical reactions, reactants or substrates undergo change as bacterial cells degrade them. As the substrates are degraded, energy is released and new compounds (products) are formed (Equation 8.2).

$$\text{substrates} \rightarrow \text{products} \tag{8.2}$$

Some of the energy released by the substrates is captured by the bacterial cells and stored in high-energy phosphate bonds for use in cellular activity. Energy that is not captured by the bacterial cells is lost to the environment as heat. New bacterial cells and carbon dioxide are products of biochemical reactions that involve organic compounds (Equation 8.3).

$$\text{substrates (organic compounds)} \rightarrow \text{bacterial cells + carbon dioxide} \tag{8.3}$$

Catalysts are involved in biochemical reactions. These catalysts are known as enzymes. Enzymes are large proteinaceous molecules that greatly accelerate the

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TABLE 8.1 The Three Stages of Anaerobic Digestion of Solids

Stage	Activity	Enzymes Used
First	Hydrolysis: Solubilization of particulate and colloidal wastes	Exoenzymes
Second	Acid forming: Conversion of soluble organic acids and alcohols to acetate, carbon dioxide, and hydrogen	Endoenzymes
Third	Methanogenesis: Production of methane and carbon dioxide	Endoenzymes

rate of biochemical reactions. However, enzymes, unlike chemical catalysts, are not altered or consumed during the reaction (Equation 8.4).

$$\text{substrates — bacteria and bacterial enzymes} \rightarrow \text{bacterial cells + carbon dioxide} \quad (8.4)$$

During some biochemical reactions, intermediate products or "intermediates" are formed (Equation 8.5). Intermediates usually are short-lived, that is, they do not accumulate. However, specific environmental or operational conditions such as a change in pH or temperature may permit the accumulation of intermediates. The presence of some intermediates may result in operation problems in an anaerobic digester.

$$\text{substrates — intermediates} \rightarrow \text{bacterial cells + carbon dioxide} \quad (8.5)$$

Initial substrates for bacteria in municipal anaerobic digesters include carbohydrates, lipids, and proteins. These substrates are found as particulates such as the carbohydrate cellulose and as colloids such as proteins.

The degradation process or digestion of solids within an anaerobic digester consists of three stages (Table 8.1). The first stage is the hydrolysis of particulate and colloidal wastes to soluble wastes in the form of organic acids and alcohols. The second stage is the conversion of the organic acids and alcohols to acetate, carbon dioxide, and hydrogen. The third stage is the production of gases, mostly methane, and new bacterial cells or sludge from acetate and hydrogen. Because a great diversity of bacteria are required in an anaerobic digester to perform hydrolysis, produce acetate and hydrogen, and produce methane, the substrate feed to the digester should contain a great diversity of wastes.

The net results of anaerobic digestion of solids are significant decreases in percent solids and percent volatile solids in digester sludge. The first and second stages of anaerobic digestion are achieved through the activities of facultative anaerobes and anaerobes, whereas the third stage is achieved through the activity of only anaerobes, the methane-forming bacteria.

Hydrolysis rates for particulate and colloidal wastes vary greatly according to the waste to be degraded and the operational conditions at the time of hydrolysis. Substrates hydrolyzed in the first stage consist of carbohydrates, lipids, and proteins. These substrates may be wasted to the digester from primary and secondary sludges.

CARBOHYDRATES

Carbohydrates are synthesized in the green leaves of plants by the conversion of carbon dioxide into glucose during photosynthesis. Carbohydrates are macromolecules or polymers that contain numerous monomers of sugars (Figure 8.1). The range of lengths of the polymers or carbohydrates varies greatly.

Within the digester all carbohydrates are degraded inside the cell of facultative anaerobes and anaerobes. Carbohydrates too large to enter the cell, that is, in an insoluble or complex soluble form, must be hydrolyzed into smaller, soluble sugars

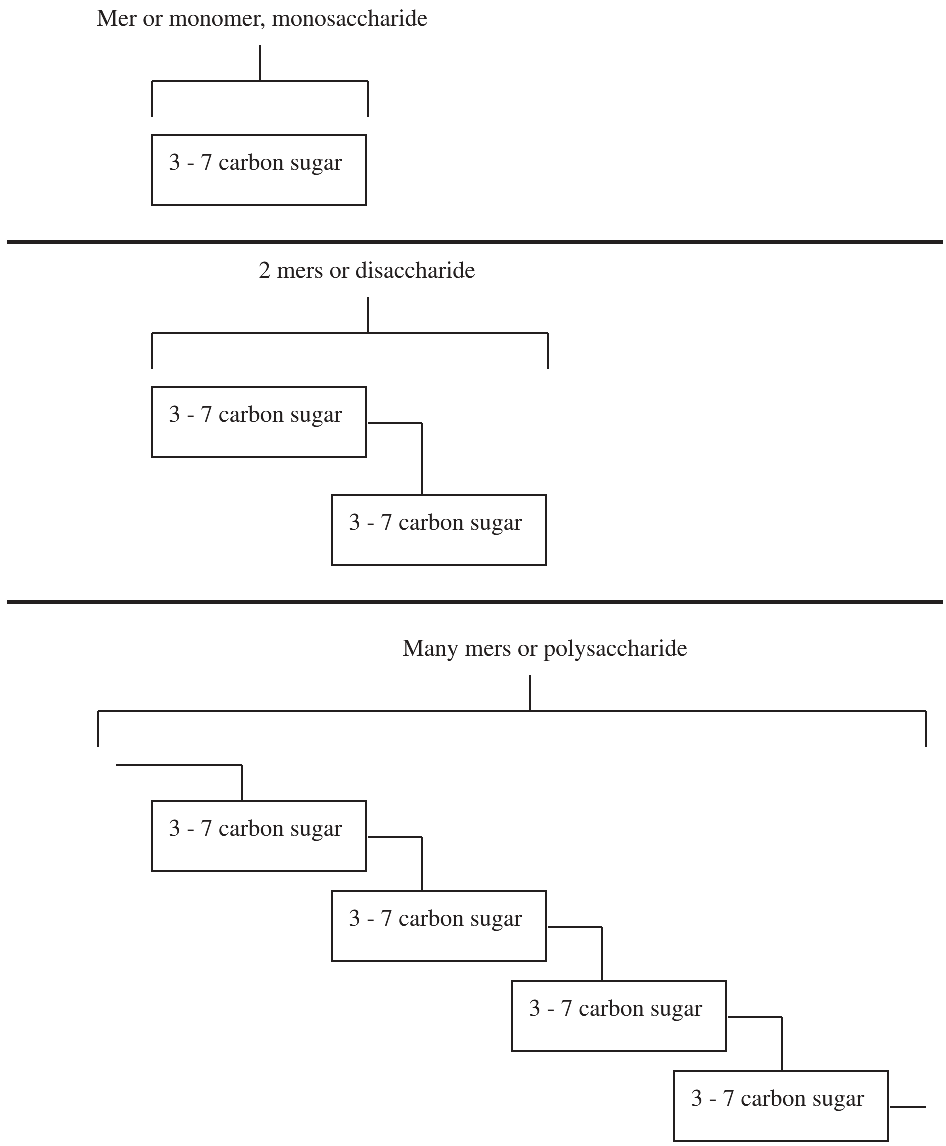


Figure 8.1

outside the cell through the use of exoenzymes (Figure 8.2). Once hydrolyzed, the smaller, soluble sugars enter the cell, where they are degraded by endoenzymes.

The monomers of carbohydrates are simple sugars (Table 8.2). These sugars are known as monosaccharides, and they contain three to seven carbon units. The common generic formulae for monosaccharides are $(CH_2O)_3 - (CH_2O)_7$. The major monomers or monosaccharides in our diet are fructose and glucose. Although many

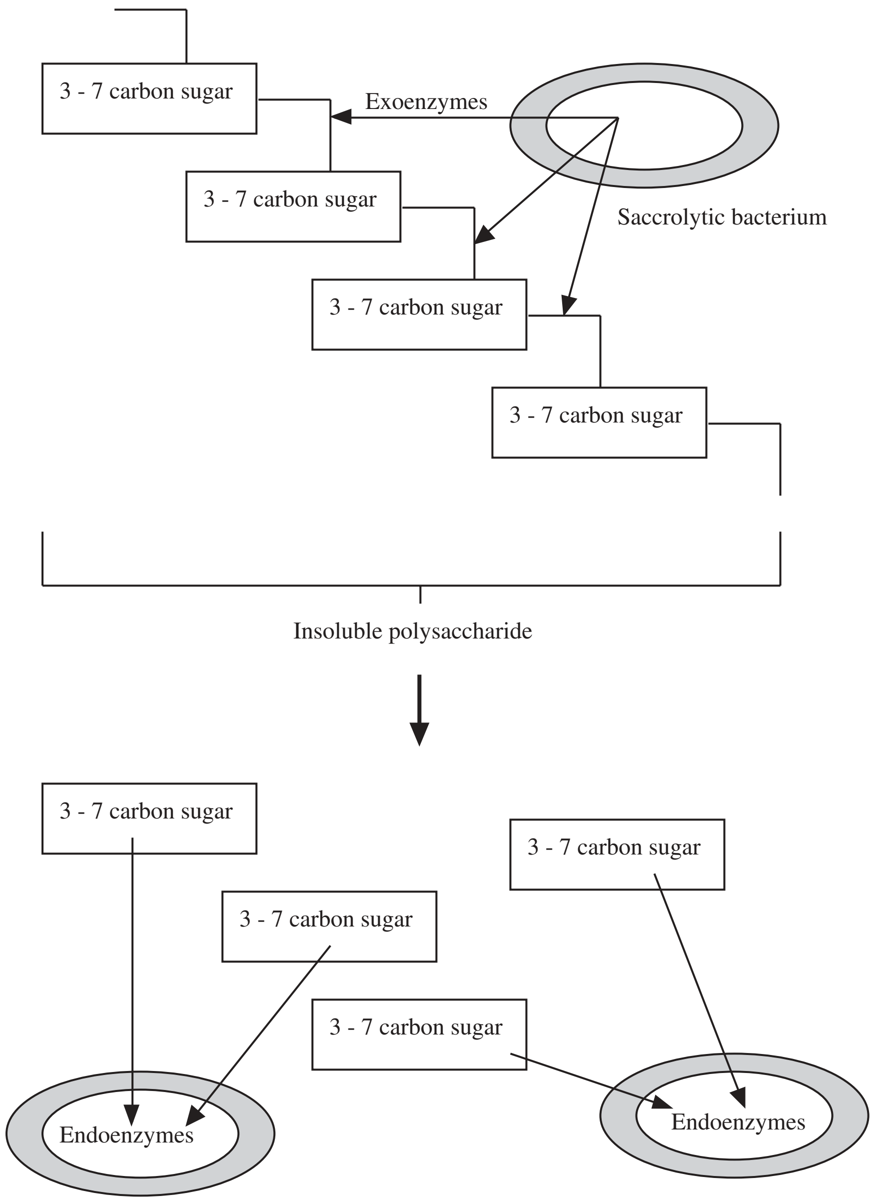


Figure 8.2 *Large, complex, and insoluble carbohydrates or polysaccharides must be hydrolyzed by sacchrolytic bacteria with the use of exoenzymes. Once solublized, the individual sugar units of the polysaccharides can enter the bacterial cells and can be degraded by endoenzymes.*

TABLE 8.2 Common Monosaccharides or Simple Sugars

Monosaccharide	Carbon Units	Formula
Deoxyribose	5	$C_5H_{10}O_5$
Glucose (dextrose)	6	$C_6H_{12}O_6$
Galactose	6	$C_6H_{12}O_6$
Fructose (levulose)	6	$C_6H_{12}O_6$
Ribose	5	$C_5H_{10}O_5$
Mannose	6	$C_6H_{12}O_6$

TABLE 8.3 Common Disaccharides

Disaccharide	Composition of Monosaccharides
Lactose	Galactose-Glucose
Maltose	Glucose-Glucose
Sucrose	Glucose-Fructose
Cellobiose	Galactose-Galactose

TABLE 8.4 Common Polysaccharides

Agar
Amylopectin (starch)
Amylose (starch)
Cellulose
Fiber
Glycogen
Pectin
Vegetable gum

monomers have identical chemical formulae, for example, glucose ($C_6H_{12}O_6$) and fructose ($C_6H_{12}O_6$), they are structurally different (Figure 8.3).

When two monomers are linked together, disaccharides are formed (Table 8.3), and when numerous monosaccharides are linked together, polysaccharides are formed (Table 8.4). Major disaccharides in our diet are sucrose (table sugar) and lactose (milk sugar). Disaccharides are carbohydrates composed of monosaccharides linked by an acetal bond (–C–O–C–). Polysaccharides often are referred to as complex carbohydrates. The largest digestible polysaccharide in our diet is starch. This polysaccharide is found in grains such as wheat and rice, root vegetables such as potatoes, and legumes such as beans and peas.

Although some sugars contain nitrogen and phosphorus, all sugars contain carbon, hydrogen, and oxygen. The basic chemical formula for sugars is $(CH_2O)_x$. The word "carbohydrate" was used originally to describe glucose—the hydrate of carbon (CH_2O) or $C_6(H_2O)_6$.

Monosaccharides are water soluble and are quickly and easily transported across the cell wall and cell membrane into the bacterial cell. Disaccharides also are water soluble but must be converted or hydrolyzed to monosaccharides before they can enter the bacterial cell.

Polysaccharides are very large, complex insoluble sugars that have a high molecular weight. These sugars require the presence of specific exoenzymes and, usually,

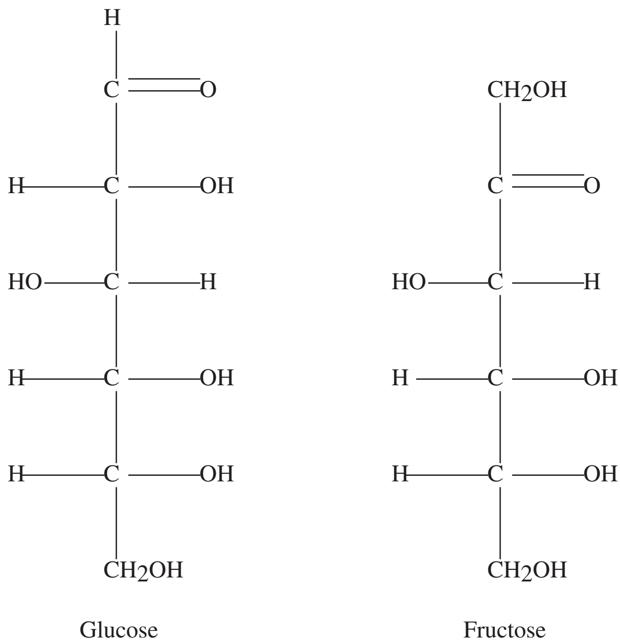


Figure 8.3

TABLE 8.5 Acids and Alcohols Produced from Monosaccharide Degradation

Genus	End Product from Monosaccharide Degradation
Clostridium	Butanol, butyrate, isopropanol
Enterobacter	Butanediol, ethanol, formate, lactate
Escherichia	Acetate, ethanol, lactate, succinate
Lactobacillus	Lactate
Streptococcus	Lactate
Propionibacterium	Acetate, propionate

several enzymatic steps to ensure their hydrolysis and degradation. Because of their insoluble nature, complex structure, and need for specific exoenzymes and numerous enzymatic steps, polysaccharides are degraded slowly.

When disaccharides and polysaccharides are hydrolyzed, monosaccharides are released. When monosaccharides are degraded in an anaerobic digester, organic acids and alcohols are produced (Table 8.5). Many of these compounds are further degraded to volatile acids.

LIPIDS

Lipids are naturally occurring organic molecules found in animal and plant tissues. Lipids do not dissolve in water, that is, lipids are extracted from animal and plant tissues with nonpolar organic solvents such as ether.

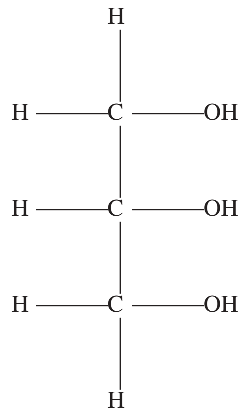


Figure 8.4

$CH_3(CH_2)_{14}COOH$
saturated palmitic acid

$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
unsaturated oleic acid

Figure 8.5

TABLE 8.6 Some Common Fatty Acids

Name	Carbon Units	Saturated	Unsaturated	Number of Double Bonds
Lauric	12	X		0
Myristic	14	X		0
Palmitic	16	X		0
Stearic	18	X		0
Linoleic	18		X	2
Linolenic	18		X	3
Oleic	18		X	1

There are numerous groups of lipids. The lipids most often wasted to a municipal anaerobic digester include fats and oils. These compounds are derived from glycerol (Figure 8.4). Glycerol combined with three fatty acids produces a triglyceride or fat.

Fatty acids are straight-chain carbon compounds containing a terminal carboxylic acid group (–COOH) (Figure 8.5). There are approximately 40 naturally occurring fatty acids (Table 8.6). Fatty acids without a double bond (=) between carbon units are known as saturated fatty acids, for example, palmitic acid. Fatty acids with a double bond between carbon units (–C=C–) are known as unsaturated fatty acids, for example, oleic acid. Fatty acids with two or more double bonds between carbon

TABLE 8.7 Composition of Some Common Fats and Oils

Fat/Oil	Animal Fat	Vegetable Oil	Principle Saturated Fatty Acid	Principle Unsaturated Fatty Acid
Butter	X		Palmitic	Oleic
Lard	X		Palmitic	Oleic
Corn		X	Palmitic	Oleic
Olive		X	Palmitic, stearic	Oleic
Peanut		X	Palmitic	Oleic
Soybean		X	Palmitic	Linoleic

units are known as polyunsaturated fatty acids, for example, linoleic acid. Palmitic acid and stearic acid are the most abundant saturated fatty acids, and oleic acid and linoleic acid are the most abundant unsaturated fatty acids.

Animal fats and vegetable fats or oils are the most abundant lipids in nature. Examples of animal fats include butter and lard, and examples of vegetable oils include corn, olive, peanut, soybean, and sunflower oils. All fats and oils have similar chemical structures. They are triglycerides. The three fatty acids of a triglyceride are not necessarily the same (Table 8.7).

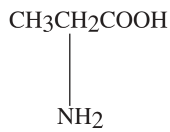
Large and complex fatty acids, fats, and oils are hydrolyzed in an anaerobic digester. The resulting small and simplistic molecules obtained from hydrolysis are degraded further to organic acids.

In anaerobic digesters fats undergo degradation through two principal steps. First, the fats are hydrolyzed to glycerol and fatty acids. Lipase enzymes are used by bacteria to hydrolyze the fats. Glycerol is degraded, and the fatty acids released through hydrolysis are degraded two carbon units at a time.

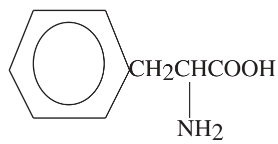
PROTEINS

The principal nitrogenous wastes in municipal sludges are proteins. Proteins are complex, high molecular-weight compounds. These molecules have a relatively large surface area and do not dissolve in wastewater or settle out of wastewater. Proteins are made of amino acids that are either straight-chain (aliphatic) or ring-shaped (cyclic) in structure (Figure 8.6). There are 20 different amino acids. Regardless of their structure, all amino acids contain an amino group ($-NH_2$) and a carboxyl group ($-COOH$). The carboxyl group is the "acid" portion of the amino acid.

Amino acids are joined together by peptide bonds to form proteins (Figure 8.7). Proteins consist of long chains of amino acids. Each protein has a unique composition and sequence of amino acids in its chain. The complex proteins formed from peptide bonds cannot be transported into bacterial cells. The use of exoenzymes, namely, proteases or peptidases, by bacteria to hydrolyze peptide bonds permits the release of individual amino acids that are transported into bacteria cells (Figure 8.8). Once inside the cell, amino acids undergo additional degradation resulting in the production of organic acids. Examples of amino acids fermented in anaerobic digesters include alanine (Equation 8.6), arginine, glutamate, glycine, and lysine (Table 8.8).



Alanine (aliphatic amino acid)



Phenylalanine (cyclic amino acid)

Figure 8.6

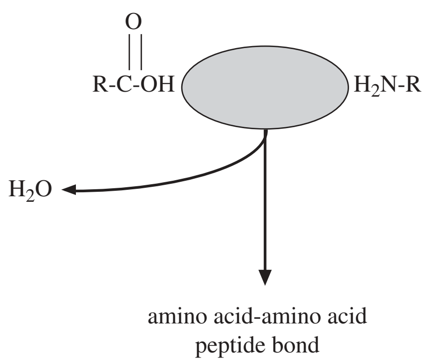


Figure 8.7 *Amino acids are joined together to form proteins. Amino acids are joined together through the production of peptide bonds. The bonds are produced by joining the hydroxyl group (–OH) in the carboxyl group (–COOH) of one amino acid with the amino group (–NH_2) of other amino acid. When the peptide bond is formed, water is produced.*

TABLE 8.8 Amino Acids Commonly Fermented in Anaerobic Digesters

Amino Acid	Fermenting Bacteria
Alanine	*Clostridium propionicium*
Arginine	*Clostridium* spp.
	Streptococcus spp.
Glutamate	*Clostridium tetanomorphium*
Glycine	*Peptostreptococcus micros*
Lysine	*Clostridium sticklandii*

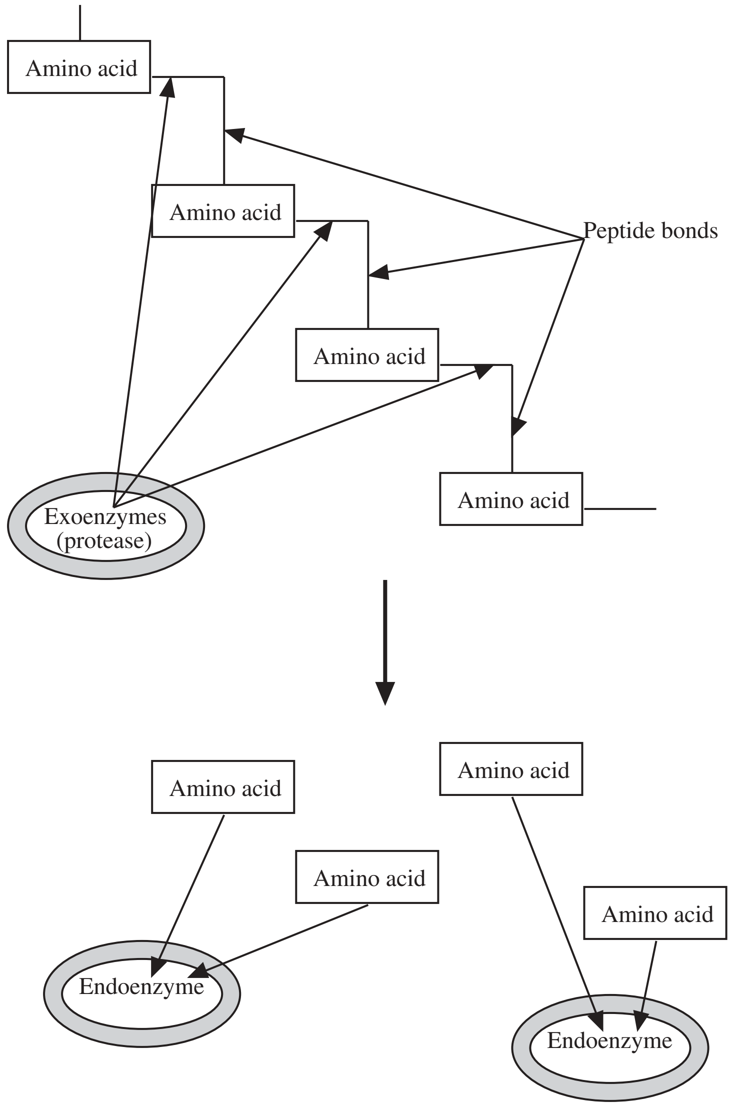


Figure 8.8 *Large, complex, and colloidal proteins must be hydrolyzed by bacteria with the use of exoenzymes. Once solublized, the individual amino acids of the proteins can enter the bacterial cells and can be degraded by endoenzymes.*

$$4H_2NCH_2COOH + 2H_2O \rightarrow 4NH_3 + 2CO_2 + 3CH_3COOH \quad (8.6)$$

The degradation of amino acids results in the production of a variety of organic acids including acetate and butyrate. Ammonia is released during the degradation of amino acids. Acetate and butyrate serve as substrates for methane-forming bacteria, whereas ammonia increases digester alkalinity or may contribute to ammonia toxicity.

Proteins can be classified as simple or conjugated according to their chemical composition. Simple proteins are those that release only amino acids and no other compounds on hydrolysis. Blood serum albumin is an example of a simple protein. Conjugated proteins are more common than simple proteins and release amino acids and non-protein substances on hydrolysis. Glycoproteins that contain carbohydrates and lipoproteins that contain lipids are examples of conjugated proteins.

VOLATILE ACIDS

Some organic acids are known also as volatile acids or volatile fatty acids. These acids occur as substrates and products in the anaerobic digester. Many serve as substrate for methane-forming bacteria, and they are the products of the fermentative activities of facultative anaerobes and anaerobes.

Volatile acid production in an anaerobic digester results in the production of methane. Although volatile acids vary in length, most volatile acids produced in an anaerobic digester are low-molecular-weight, short-chain acids, for example, formate (1 carbon unit), acetate (2 carbon units), propionate (3 carbon units), and butyrate (4 carbon units) (Table 8.9).

These short-chain acids are known as volatile acids because they can vaporize or evaporate at atmospheric pressure. Of these acids, acetate is the predominant acid produced in an anaerobic digester. Approximately 85% of the volatile acid content of an anaerobic digester is acetate. All volatile acids are soluble in water

As wastes are degraded, new bacterial cells or sludge are produced. The cellular growth or amount of sludge produced is expressed as net biomass yield [as percentage of chemical oxygen demand (COD) removed]. Growth yield for several wastes is presented in Table 8.10.

TABLE 8.9 Volatile Acids Commonly Found in Anaerobic Digesters

Volatile Acid	Number of Carbon Units	Formula
Formate	1	$HCOOH$
Acetate	2	CH_3COOH
Propionate	3	CH_3CH_2COOH
Butyrate	4	$CH_3(CH_2)_2COOH$
Valeric acid	5	$CH_3(CH_2)_3COOH$
Isovaleric acid	5	$(CH_3)_2CHCH_2COOH$
Caproic acid	6	$CH_3(CH_2)_4COOH$

TABLE 8.10 Growth Yields (as % of COD removed)

Substrate (waste)	Yield
Alcohols	0.06–0.08
Carbohydrates	0.08–0.15
Organic acids	0.02–0.04
Proteins	0.03–0.06

The higher the volatile solids feed to the digester, the larger the amount of volatile acids formed in the digester. The larger the amount of volatile acids in the digester, the greater the impact of volatile acids on digester alkalinity and pH. Therefore, sludges that have a high volatile content should be transferred slowly to an anaerobic digester.

9

Biogas

Anaerobic digestion of municipal sludges results in the production of a mixture of gases (Figure 9.1). Collectively, these gases are referred to as digester gas or biogas. The only gas of economic value that is produced in an anaerobic digester is methane. In a properly operating digester most of the gas produced from a day's feed sludge appears within 24 hours.

Methane can be used as a source of fuel. It is a natural flammable gas. Methane is odorless and burns cleanly (Equation 9.1). Pure methane has a heat value of 1,000 Btu/ft^3. When methane is mixed with carbon dioxide that is produced in an anaerobic digester, its heat value decreases significantly.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \qquad (9.1)$$

Typically, biogas production in municipal anaerobic digesters is between 10 and 25 ft^3 per pound of volatile solids degraded (cu ft/lb VS) or 0.75–1.0 m^3/kg VS. The heat value of biogas is approximately 500–600 Btu/ft^3, much lower than that of methane because of the dilution of methane by carbon dioxide. With increasing quantities of carbon dioxide in biogas, decreasing heat values of biogas occur. If the carbon dioxide content of biogas becomes too large, biogas will not allow for a self-sustained burn and supplemental fuel will be required. If the carbon dioxide fraction in the biogas increases above 30%, the acid concentration in the sludge increases and the pH drops below 7.0. At pH values below 7.0, significant acid fermentation occurs.

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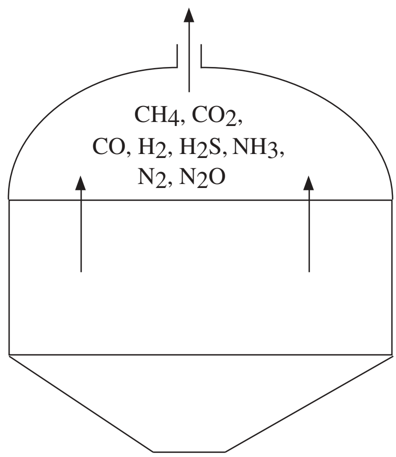


Figure 9.1

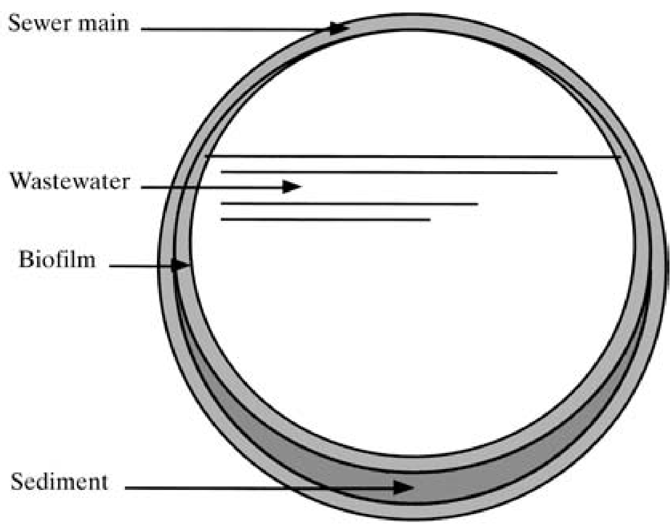


Figure 9.2 *Anaerobic respiration occurs in the sewer main. Anaerobic respiration occurs in the biofilm lining the inside of the sewer main and in the sediment.*

Numerous gases are produced in an anaerobic digester. The gases produced in largest quantities are methane and carbon dioxide. By volume, methane is 60% to 65%, and carbon dioxide is 35 to 40%. Most municipal wastewater treatment plants use biogas to heat digesters to 32–35°C (90–95°F). The biogas also may be used to heat buildings. Biogas not used to heat digesters is simply flamed.

When anaerobic digestion of sludges and wastewaters is interrupted by changes in operational conditions, numerous insoluble and volatile compounds are produced. These compounds may be released wherever anaerobic digestion of organic

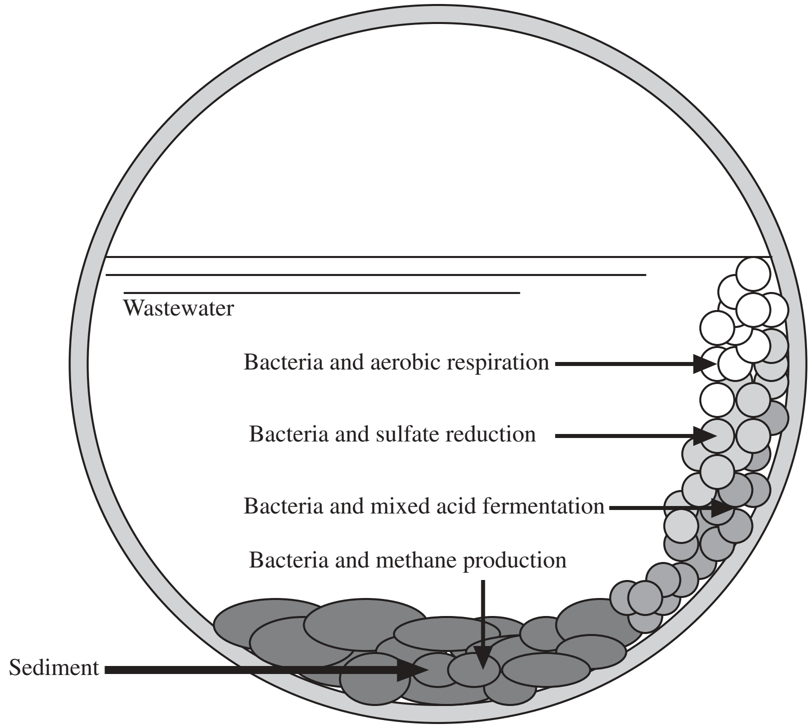


Figure 9.3 *Within the sewer main aerobic respiration and anaerobic respiration occur. Bacteria on the surface of the biofilm that are exposed to free molecular oxygen use aerobic respiration. Bacteria beneath the surface of the biofilm that do not receive free molecular oxygen use anaerobic respiration using sulfate ions or mixed acid fermentation. Bacteria at the bottom of the sediment use anaerobic respiration and produce methane. Because nitrate ions and nitrite ions are seldom found in sewer mains, anoxic respiration does not occur.*

compounds is interrupted. Many of these compounds are malodorous and often are released in sewer mains (Figures 9.2 and 9.3), lift stations, secondary clarifier sludge blanket, thickener, and anaerobic digester. The organic and inorganic compounds produced are listed in Tables 9.1 and 9.2.

The organic compounds (Table 9.1) include methane and volatile organic compounds (VOC). The VOC contain volatile fatty acids (VFA), nitrogen-containing compounds, and volatile sulfur compounds (VSC). The production of nitrogen-containing VOC and VSC is usually due to the degradation of proteinaceous wastes.

Of the inorganic gases (Table 9.2) produced in an anaerobic digester, hydrogen sulfide (H_2S) is the most undesirable. If biogas contains too much hydrogen sulfide, the gas may damage digester equipment. Hydrogen sulfide can be scrubbed from biogas, but scrubbing is expensive and often cost-prohibitive for small wastewater treatment plants. Excess production of hydrogen sulfide is due to the excess of sulfur-containing wastes such as proteinaceous compounds that are transferred to the digester.

TABLE 9.1 Organic Gases Produced Through Microbial Activity in Anaerobic Digesters

Name	Formula	VFA	VSC
Acetate	CH_3COOH	X	
Butyrate	$CH_3(CH_2)_2CH_2COOH$	X	
Caproic acid	$CH_3(CH_2)_4COOH$	X	
Formate	$HCOOH$	X	
Propionate	CH_3CH_2COOH	X	
Succinate	$CH_3CHOHCOOH$	X	
Valeric acid	$CH_3(CH_2)_3COOH$	X	
Methane	CH_4		
Cadaverine	$H_2N(CH_2)_5NH_2$		
Dimethylamine	CH_3NHCH_3		
Ethylamine	$C_3H_5NH_2$		
Indole	$C_8H_{13}N$		
Methylamine	CH_3NH_2		
Putrescine	$H_2N(CH_2)_4NH_2$		
Propylamine	$CH_3CH_2CH_2NH_2$		
Pyridine	C_5H_6N		
Skatole	C_9H_9N		
Trimethylamine	$CH_3NCH_3CH_3$		
Allyl mercaptan	$CH_2{=}CHCH_2SH$		X
Benzyl mercaptan	$C_6H_5CH_2SH$		X
Dimethyl sulfide	$(CH_3)_2S$		X
Ethyl mercaptan	C_2H_5SH		X
Methyl mercaptan	CH_3SH		X
Thiocresol	$CH_3C_6H_4SH$		X
Thioglycolic acid	$HSCH_2COOH$		X

TABLE 9.2 Inorganic Gases Produced Through Microbial Activity in Anaerobic Digesters

Name	Formula
Ammonia	NH_3
Carbon dioxide	CO_2
Carbon disulfide	CS_2
Carbon monoxide	CO
Hydrogen sulfide	H_2S
Nitrogen	N_2
Nitrous oxide	N_2O

The inorganic gases molecular nitrogen (N_2) and nitrous oxide (N_2O) are produced through anoxic respiration (denitrification) in the anaerobic digester. Anoxic respiration can occur with the transfer of nitrate ions (NO_3^-) to the digester with sludges or the addition of nitrate-containing compounds such as sodium nitrate ($NaNO_3$) to increase digester alkalinity.

Part III

Operational Conditions

10

Introduction to Operational Conditions

The rate-limiting reaction in anaerobic digestion is usually the conversion of volatile acids to methane. Methane-forming bacteria obtain very little energy from the degradation of volatile acids. Most of the energy released from the volatile acids is transferred to the methane.

Because of the low energy yield obtained from volatile acids by methane-forming bacteria, their growth rate is restricted, that is, the amount of substrate utilization per unit of organisms is high. Therefore, bacterial growth or sludge production is low and optimum operational conditions must be maintained for satisfactory rates of solids destruction and methane production. These factors are responsible for the rate-limiting reaction of the conversion of volatile acids to methane. However, if the substrates fed to the anaerobic digester were mostly slowly degrading particulate materials, then the rate-limiting reaction would be the hydrolysis of the particulate material.

Methane-forming bacteria are strict anaerobes and are extremely sensitive to changes in alkalinity, pH, and temperature. Therefore, operational conditions in the digester must be periodically monitored and maintained within optimum ranges. In addition to alkalinity, pH, and temperature, several other operational conditions should be monitored and maintained within optimum ranges for acceptable activity of methane-forming bacteria. These conditions are gas composition, hydraulic retention time (HRT), oxidation-reduction potential (ORP), and volatile acid concentration (Table 10.1).

Process control of anaerobic digesters is often difficult, because numerous operational conditions are interrelated and changes in one condition may directly or indirectly affect others. Also, the relatively low concentrations of solids and short solids retention times (SRTs) maintained in completely mixed digesters render the

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TABLE 10.1 Operational Conditions for Acceptable Activity of Methane-forming Bacteria and Methane Production

Condition	Optimum	Marginal
Alkalinity, mg/l as $CaCO_3$	1500–3000	1000–1500 3000–5000
Gas composition		
Methane, % volume	65–70	60–65 & 70–75
Carbon dioxide, % volume	30–35	25–30 & 35–40
Hydraulic retention time, days	10–15	7–10 & 15–30
pH	6.8–7.2	6.6–6.8 & 7.2–7.6
Temperature, mesophilic	30–35°C	20–30° & 35–40°C
Temperature, thermophilic	50–56°C	45–50° & 57–60°C
Volatile acids, mg/l as acetic acid	50–500	500–2000

process susceptible to toxic upsets and shock loadings. Another difficulty in achieving proper digester operation is the presence of different bacterial groups that have different optimum values or ranges of values for operational conditions. For example, there are two optimal temperatures for anaerobic digestion of solids. The acid-forming bacteria have an optimum temperature at 30°C, and the mesophilic, methane-forming bacteria have an optimum temperature at 35°C.

11

Start-up

Primary and secondary sludges that provide the substrates for an anaerobic digester also provide the bacteria needed for the hydrolysis and degradation of these compounds and the production of methane. Both facultative anaerobes and anaerobes including methane-forming bacteria are needed in an anaerobic digester. Facultative anaerobes and anaerobes are needed for 1) the hydrolysis of particulate and colloidal compounds and 2) the degradation of soluble organic compounds to volatile acids. Methane-forming bacteria are needed for the degradation of volatile acids and the production of methane.

To seed an anaerobic digester with an adequate population of facultative anaerobes and anaerobes including methane-forming bacteria, a ratio of 1:10 of secondary sludge to primary sludge may be used. Although the amount of secondary sludge is much less compared with primary sludge, the secondary sludge is highly concentrated with facultative anaerobes. The primary sludge provides not only some facultative anaerobes but also many anaerobes including methane-forming bacteria and many organic particulates.

Because methane-forming bacteria are strict anaerobes and die quickly in an activated sludge process, an anaerobic digester cannot be successfully seeded with secondary sludge alone. Therefore, primary sludge that contains an abundant population of methane-forming bacteria is needed. Primary sludge performs three important roles during anaerobic digester start-up. These roles consist of seeding the digester with 1) methane-forming bacteria, 2) facultative anaerobes and anaerobes, and 3) organic particulates.

Once an anaerobic digester has been seeded properly and is operating efficiently, the digester can be fed secondary sludge alone. Secondary sludge contains numerous facultative anaerobes and many particulate and colloidal organics. Primary

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sludge contains only a relatively small number of facultative anaerobes that would not adequately replace the bacteria wasted from the digester during routine solids pumping and dewatering operations.

Because the successful operation of an anaerobic digester requires the activity of an abundant and diverse population of methane-forming bacteria, seeding the digester that is heated to 35°C with fresh cow manure may be helpful. Seeding with

TABLE 11.1 Chemicals Commonly Used for pH Adjustment and Alkalinity Addition

Chemical	Formula
Ammonia, anhydrous	NH_3
Caustic soda	NaOH
Lime, quick	CaO
Lime, hydrated	$Ca(OH)_2$
Soda ash	Na_2CO_3
Sodium bicarbonate	$NaHCO_3$

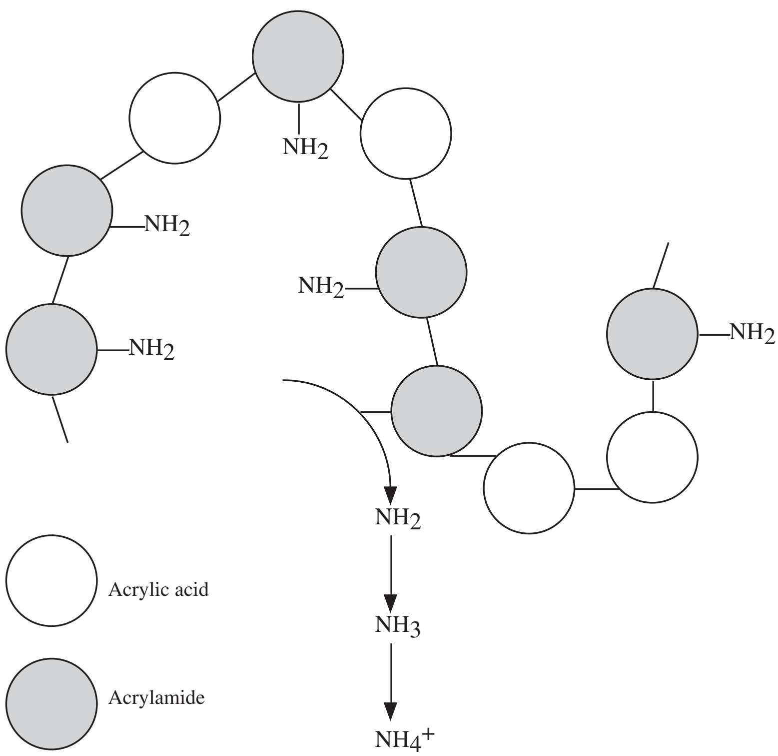


Figure 11.1 Cationic, polyacrylamide polymers are commonly used at wastewater treatment plants for solids capture and sludge thickening and dewatering. Often these polymers may be found in relatively large concentrations in anaerobic digesters. The degradation of the polymers results in the release of amino groups from the acrylamide component of the polymer. The amino group is quickly converted to ammonia and then ammonium ions in the anaerobic digester. The conversion of ammonia to ammonium ions is pH dependent.

cow manure can be practiced during start-up or when the efficiency of the digester deteriorates, for example, when the digester goes "sour." Approximately 5 gallons of fresh cow manure should be added to the digester for every 100,000 gallons of digester sludge. The manure should be added daily until successful start-up or improved efficiency is obtained.

Numerous methane-forming bacteria are alive and active deep within cow manure. Care should be taken not to expose the bacteria within the cow manure to the atmosphere. Methane-forming bacteria die quickly in the presence of free molecular oxygen. Difficulties during start-up of an anaerobic digester also can be overcome by inoculating a digester with previously digested sludge.

During start-up, loading to the digester should proceed slowly. Careful monitoring and control of pH and alkalinity are essential. This is especially true when good seed is not available. The digester pH should be maintained within the optimum range of 6.8 to 7.2. The pH within this range is required for acceptable activity of

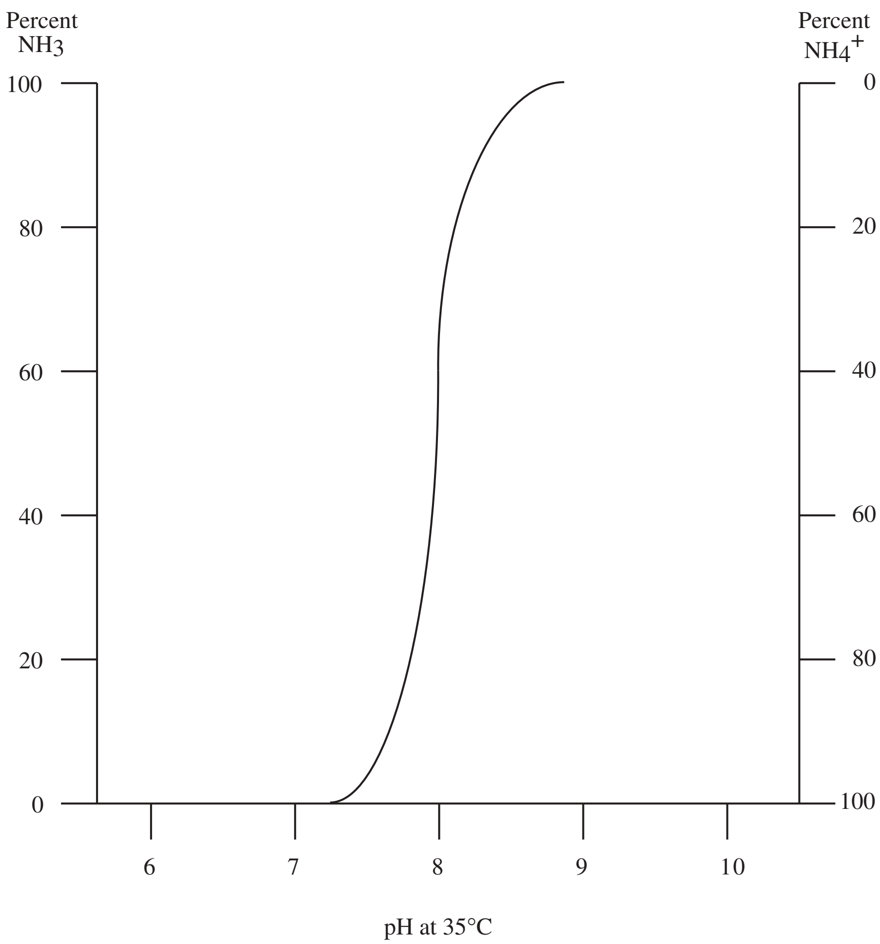


Figure 11.2 *The quantities of the reduced forms of nitrogen—ammonia and ammonium ion—in an anaerobic digester are pH dependent. Increasing pH results in the production of more ammonia, while decreasing pH results in the production of more ammonium ions.*

methane-forming bacteria; it also helps to ensure that adequate buffering capacity or alkalinity is present to neutralize the acids within the digester.

Anaerobic digester start-up should proceed smoothly, and the time between initial digester feed sludge and stable operation should be as short as possible. Approximately 1 month will be required to achieve a steady-state condition or an efficiently operating digester. This condition is reflected by the production of burnable biogas and a stable volatile acid-to-alkalinity ratio.

Several chemicals can be added to an anaerobic digester to maintain proper pH and alkalinity (Table 11.1). The choice of chemical is dependent on cost, handling, safety, storage, and requirements for feeding the chemical to the digester. If the pH within the digester is greater than the optimum range, ammonia toxicity may occur.

Ammonium ions (NH_4^+) are a natural component of a municipal anaerobic digester. The ions are produced in the digester as a result of bacterial degradation of amino acids and proteins. Ammonium ions may be added to the digester in secondary sludge as a result of protein degradation or in primary and secondary sludges that contain cationic polyacrylamide polymers. These polymers contain amino groups ($–NH_2$) that are released through bacterial activity. Once released, these groups form NH_4^+ (Figure 11.1).

Ammonia in the digester may be in the form of ammonium ions (ionized ammonia—NH_4^+) or dissolved ammonia gas (nonionized ammonia—NH_3). The two forms are in equilibrium, and the relative concentration of each form is dependent on the digester pH (Figure 11.2). When the digester pH is 7.2 or lower, the presence of NH_4^+ is favored. When the digester pH is greater than 7.2, the presence of NH_3 is favored. Dissolved ammonia gas or NH_3 is toxic to bacteria, especially methane-forming bacteria.

Ammonia toxicity can be avoided if the digester pH is maintained within the optimum range of 6.8 to 7.2 and the ammonia-nitrogen concentration does not increase into the range of 1500 to 3000 mg/l. An additional problem related to an increase in ammonia-nitrogen or alkalinity is foam and scum production. This problem often occurs during digester start-up.

12

Sludge Feed

Because the predominant application of anaerobic digesters is the degradation of particulate and colloidal wastes, sludge feed or organic loading rates to digesters usually are expressed in terms of volatile solids (VS). Designed and recommended loadings for anaerobic digesters that are mixed and heated are 200–450 lb VS/ $1000\,ft^3$/day (3.2–7.2 kg VS/m^3/day). However, loading rates of 30–50 lb VS/ $1000\,ft^3$/day (0.5–0.6 kg VS/m^3/day) are typical. Higher loading rates could be treated if a more concentrated sludge could be fed to the digester.

The volatile solids loadings to anaerobic digesters are controlled in most wastewater treatment plants by the efficiency of the primary and secondary clarifiers in removing and concentrating sludge. Therefore, the thickening of sludge is an important operational factor affecting digester performance.

Typically, raw sludges or feed sludges having low solids content are transferred to municipal anaerobic digesters . These sludges often contain 3–6% solids. These dilute feed sludges adversely impact digester operation. They reduce hydraulic retention time (HRT), reduce volatile solids destruction, and reduce methane production.

The blending of primary and secondary sludges may be helpful in improving anaerobic digester performance (Figure 12.1). Primary sludge may be blended with thickened excess activated sludge, or blended primary and secondary sludges may be thickened.

The percentage of primary sludge in feed sludge may influence VS reduction in the anaerobic digester. Generally, with increasing percent primary sludge in digester feed sludge, an increase in VS reduction can be expected.

The HRT of anaerobic digesters is affected by not only the quantity of feed sludge but also the quantities of digested sludge, supernatant, and grit. Digested

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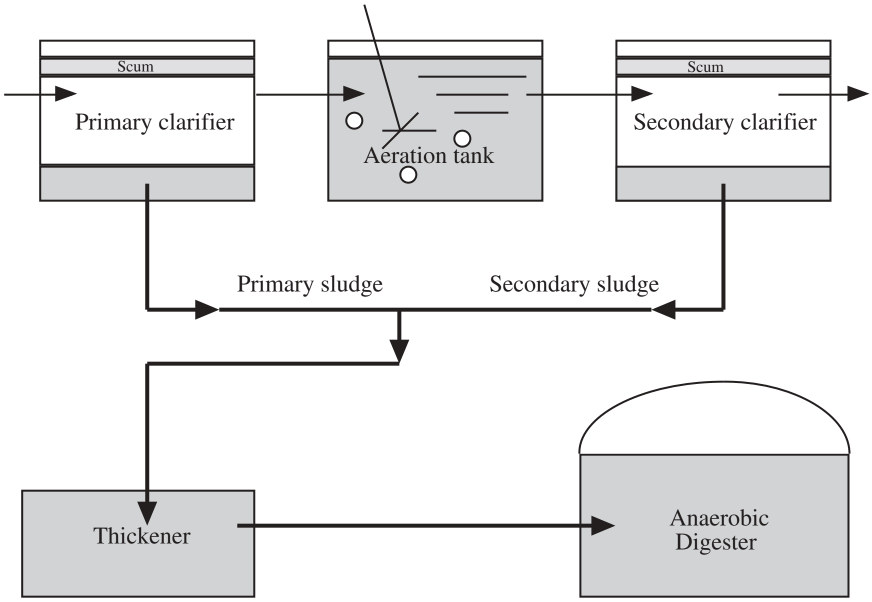


Figure 12.1 *Digester performance or treatment efficiency is affected by the blending of primary and secondary sludges.*

sludge and supernatant must be withdrawn on a routine basis, and grit must be removed as needed to ensure adequate retention time. Common operational problems associated with anaerobic digesters are overpumping of raw sludge and excessive withdrawal of digested sludge.

13

Retention Times

There are two significant retention times in an anaerobic digester. These are solids retention time (SRT) and hydraulic retention time (HRT). The SRT is the average time that bacteria (solids) are in the anaerobic digester. The HRT is the time that the wastewater or sludge is in the anaerobic digester. The SRT and the HRT are the same for a suspended-growth anaerobic digester that has no recycle. If recycle of solids is incorporated in the operation of the digester, then the SRT and HRT may vary significantly.

Because the generation time, that is, the time required for a population of bacteria to double in size, of methane-forming bacteria is relatively long compared with aerobic bacteria and facultative anaerobic bacteria (Table 13.1), typical SRTs for anaerobic digesters are >12 days. Detention times <10 days are not recommended. At detention times <10 days significant washout of methane-forming bacteria occurs. This indicates that SRT, not HRT, is the more important retention time. The SRT is not greatly affected by the nature of the wastewater or sludge under treatment, unless the wastewater or sludge is toxic to the bacteria.

Anaerobic digesters that utilize fixed-film media for the growth of bacteria favor the development of a concentrated mass (biomass) of bacteria that are attached to the media. The biomass prevents the washout of large numbers of bacteria and permits high SRT values.

High SRT values are advantageous for anaerobic digesters. High SRT values maximize removal capacity, reduce required digester volume, and provide buffering capacity for protection against the effects of shock loadings and toxic compounds in wastewaters and sludges. High SRT values also help to permit biological acclimation to toxic compounds. High SRT values may be achieved through two measures. First, the volume of the digester may be increased. Second, the concentration of the bacteria (solids) may be increased.

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TABLE 13.1 Approximate Generation Times of Important Groups of Wastewater Bacteria

Bacterial Group	Function	Approximate Generation Time
Aerobic organotrophs	Floc formation and degradation of soluble organics in the activated sludge and trickling filter processes	15–30 min
Facultative anaerobic organotrophs	Floc formation and degradation of soluble organics in the activated sludge and trickling filter processes, hydrolysis and degradation of organics in the anaerobic digester	15–30 min
Nitrifying bacteria	Oxidation of NH_4^+ and NO_2^- in the activated sludge and trickling filter processes	2–3 days
Methane-forming bacteria	Production of methane in the anaerobic digester	3–30 days

The conversion of volatile solids to gaseous products in an anaerobic digester is controlled by the HRT. The design of the HRT is a function of the final disposition of the digested sludge. The HRT may be relatively high or low, if the digested sludge is to be land applied or incinerated, respectively. However, increases in detention time >12 days do not contribute significantly to increased destruction of volatile solids.

HRT values affect the rate and extent of methane production. Of all the operational conditions within an anaerobic digester, for example, temperature, solids concentration, and volatile solids content of the feed sludge, HRT is perhaps the most important operational condition affecting the conversion of volatile solids to gaseous products.

14

Temperature

Common recurring problems associated with anaerobic digesters are loss of heating capability and maintenance of optimum digester temperature. An acceptable and uniform temperature should be maintained throughout the digester to prevent localized pockets of depressed temperature and undesired bacterial activity. Variations in temperature of even a few degrees affect almost all biological activity including the inhibition of some anaerobic bacteria, especially methane-forming bacteria. Adequate mixing of the digester content prevents the development of localized pockets of temperature variation.

Most methane-forming bacteria are active in two temperature ranges. These ranges are the mesophilic range from 30 to 35°C and the thermophilic range from 50 to 60°C. At temperatures between 40°C and 50°C, methane-forming bacteria are inhibited. Digester performance falters somewhere near 42°C, as this represents the transition from mesophilic to thermophilic organisms.

Although methane production can occur over a wide range of temperatures (Figure 14.1), anaerobic digestion of sludge and methane production at municipal wastewater treatment plants is performed in the mesophilic range, with an optimum temperature of approximately 35°C (Table 14.1).

Whenever digester temperature falls below 32°C, close attention should be paid to the volatile acid-to-alkalinity ratio. Volatile acid formation continues at depressed temperatures, but methane production proceeds slowly. Volatile acid production can continue at a rapid rate as low as 21°C, whereas methane production is essentially nonexistent. Therefore, 32°C is the minimum temperature that should be maintained, and 35°C is the preferred temperature.

Although methane-forming bacteria are active and grow in several temperature ranges (Table 14.2), most methane-forming bacteria are mesophiles. Some

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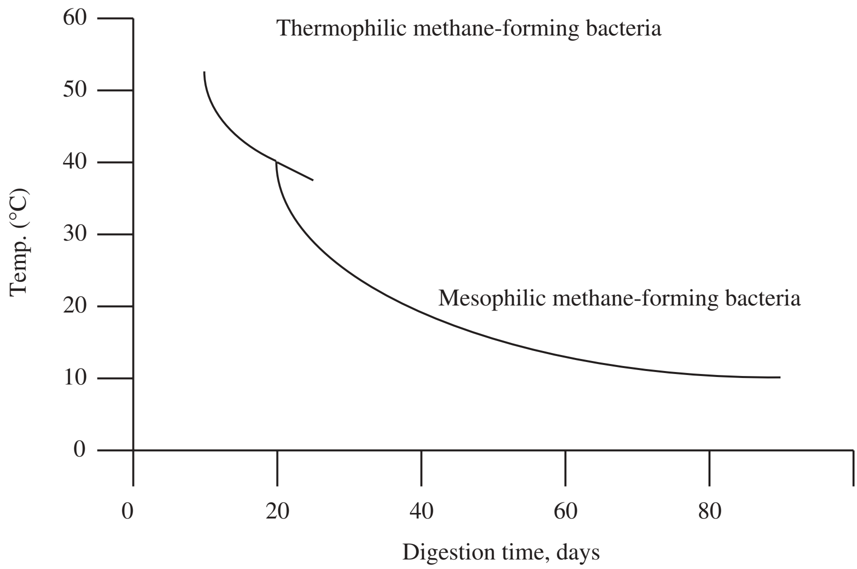


Figure 14.1 *Methane production occurs over a relatively large range of temperature values. Most anaerobic digesters, especially those at municipal wastewater treatment plants, operate in the mesophilic range of temperatures.*

TABLE 14.1 Temperature Range for Methane Production for Municipal Anaerobic Digesters

Temperature, °C	Methane Production
35	Optimum
32–34	Minimum
21–31	Little, digester going "sour"
<21	Nil, digester is "sour"

TABLE 14.2 Optimum Temperature Ranges for the Growth of Methane-forming Bacteria

Bacterial Group	Temperature Range, °C
Psychrophiles	5–25
Mesophiles	30–35
Thermophiles	50–60
Hyperthermophiles	>65

methane-forming bacteria are psychrophiles, thermophiles, and hyperthermophiles or stearothermophiles. Anaerobic sludge digestion in the psychrophilic range usually is confined to small-scale treatment units such as Imhoff tanks, septic tanks, and sludge lagoons. Here the digestion process is not heated, and the temperature of the digester sludge is approximately equal to the outside environment. Therefore, the rate of digestion of sludge varies from season to season. Because of the

TABLE 14.3 Comparison of Mesophilic and Thermophilic Digesters

Feature	Mesophilic Digester	Thermophilic Digester
Loading rates	Lower	Higher
Destruction of pathogens	Lower	Higher
Sensitivity to toxicants	Lower	Higher
Operational costs	Lower	Higher
Temperature control	Less difficult	More difficult

depressed temperature of the digester sludge, the sludge retention time (SRT) is usually long, often greater than 12 weeks.

Methane production in the thermophilic range is usually performed at industrial wastewater treatment plants that are able to heat wastewaters or sludges. A comparison of advantages and disadvantages of mesophilic and thermophilic digesters is presented in Table 14.3. The greater destruction of pathogens by thermophilic digesters has drawn attention to their use to satisfy existing and proposed regulations for the disposal and reuse of municipal sludges.

The rate of anaerobic digestion of sludge and methane production is proportional to digester temperature, that is, the higher the temperature the greater the destruction rate of volatile solids and the production of methane. The rate of anaerobic digestion of sludge and methane production is considerably faster in thermophilic digesters than in mesophilic digesters.

Although 25% to 50% more activity occurs in thermophilic digesters than in mesophilic digesters, there are several significant microbiological characteristics associated with thermophilic anaerobes and thermophilic digestion that may adversely affect digester performance. These characteristics include 1) the low bacterial growth or yield (increase in population size) of these anaerobes, 2) the high endogenous death rates of these bacteria, and 3) the lack of diversity of these anaerobes. These characteristics are responsible for 1) relatively high residual values of volatile acids, for example, >1000 mg/l, and 2) inconsistent treatment of sludge during continuously shifting operational conditions. Also, thermophilic anaerobes are very sensitive to rapid changes in temperature. Therefore, fluctuations in digester temperature should be as small as possible, that is, <1°C per day for thermophiles and 2–3°C per day for mesophiles.

Temperature is one of the most important factors affecting microbial activity within an anaerobic digester, and methane production is strongly temperature dependent. Fluctuations in temperature affect the activity of methane-forming bacteria to a greater extent than the operating temperature.

Temperature influences not only methane-forming bacteria but also volatile acid-forming bacteria. Therefore, fluctuations in temperature may be advantageous to certain groups and disadvantageous to other groups. For example, a 10°C temperature increase can stop methane production or methane-forming bacterial activity within 12 hours, while volatile acid production increases. Changes in the activity of different groups of volatile acid-forming bacteria result in changes in the relative quantities of organic acids and alcohols produced during fermentation. Changes in the quantities of organic acids and alcohols that are used directly and indirectly as substrates by methane-forming bacteria affect overall digester performance.

The effect of temperature on hydrolysis of particulate and colloidal wastes is not very great. Hydrolytic bacteria are not as sensitive to temperature change as the acetate-forming bacteria and methane-forming bacteria.

Temperature affects biological activity. This effect is due mostly to the impact of temperature on enzymatic activity or reactions. Therefore, increases in temperature result in more enzymatic activity whereas decreases in temperature result in less enzymatic activity. Because of the impact of temperature on enzymatic activity, SRT within digesters should increase with decreasing temperatures.

Although anaerobic bacteria can be acclimated to operating temperatures outside their optimum range, biomass activity and digester performance may be adversely affected. Because methane-forming bacteria grow slowly and are very sensitive to small changes in temperature, acclimation must proceed very slowly.

15

Nutrients

Although nutrient needs for bacteria in aerobic and anaerobic biological treatment processes may be grouped as macronutrients and micronutrients, there are significant differences in nutrient requirements between these two treatment processes. These differences are due to the unique needs of methane-forming bacteria and the lower cell (sludge) yield of fermentative bacteria as compared to aerobic bacteria.

Macronutrients, for example, nitrogen and phosphorus, are nutrients that are required in relatively large quantities by all bacteria. Micronutrients, for example, cobalt and nickel, are nutrients that are required in relatively small quantities by most bacteria. The inorganic nutrients critical in the conversion of acetate to methane—the rate-limiting reaction in an anaerobic digester—are the macronutrients nitrogen and phosphorus and the micronutrients cobalt, iron, nickel, and sulfur.

MACRONUTRIENTS

Macronutrient requirements for anaerobic biological treatment processes are much lower than the requirements for aerobic biological treatment processes such as activated sludge and trickling filter processes. The reduced requirement for macronutrients in anaerobic processes is due to lower cell (sludge) yield compared with aerobic processes from the degradation of equal quantities of substrate.

The two macronutrients of concern in any biological treatment process are nitrogen and phosphorus. These nutrients are made available to anaerobic bacteria, including methane-forming bacteria, as ammonical-nitrogen (NH_4^+–N) and orthophosphate-phosphorus (HPO_4^-–P). These nutrients, like all nutrients, are available to bacteria only in a soluble form.

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Although NH_4^+–N is the preferred nitrogen nutrient for methane-forming bacteria, some methane-forming bacteria can obtain nitrogen from other sources. Some are able to fix molecular nitrogen (N_2), and some are able to use the amino acid alanine (CH_3CHNH_2COOH). Orthophosphate-phosphorus is the preferred phosphorus nutrient.

The amount of nitrogen and the amount of phosphorus needed to satisfy anaerobic bacterial activity and maintain acceptable digester performance may be determined by one of two methods. The first method consists of calculating the amount of nutrients that must be present in the digester feed sludge and, if necessary, adding the nutrient. In the second method, adequate residual concentrations of soluble nutrients must be found in the digester effluent. If these residual concentrations are not found, the nutrients must be added.

Because carbonaceous biochemical oxygen demand (cBOD) is measured under aerobic conditions over a rather short test period (5 days) compared with relatively long digester retention times (>12 days), the resulting cBOD tends to underestimate the total oxygen demand present in a sample of sludge or wastewater. Also, oxygen is not used in an anaerobic digester to degrade organic compounds. Therefore, under anaerobic conditions of degradation of substrates in which oxygen is not used and hydrolysis of substrates occurs, cBOD underestimates the total strength of a sample of sludge or wastewater. These discrepancies have led to the use of chemical oxygen demand (COD) for characterization of the strength of a sample of sludge or wastewater.

The amount of nitrogen and the amount of phosphorus that must be available in the digester can be determined from the quantity of substrate or COD of the digester feed sludge. Nutrient requirements for anaerobic digesters vary greatly at different organic loading rates (Figure 15.1). Generally, COD:N:P of 1000:7:1 and 350:7:1 have been used for high-strength wastes and low loadings, respectively. These ratios have a C/N value of at least 25:1 that is suggested for optimal gas production. If either of these ratios is used, it is assumed that nitrogen is approximately 12% of the dry weight of bacterial cells or sludge and phosphorus is approximately 2% of the dry weight of bacterial cells or sludge (Table 15.1). These ratios are based on the common empirical formula for cellular material, $C_2H_7O_2N$.

By assuming that 10% of the COD fed to the digester is converted to new bacterial cells ($C_2H_7O_2N$), that is, growth yield of 0.1 kg VSS/kg COD removed, the amount of nitrogen and phosphorus that are needed can be calculated. For example, if the COD of the digester feed sludge is 10,000 mg/l, and 80% of the COD is degraded, then the amount of nitrogen and the amount of phosphorus that are needed are 96 mg/l and 16 mg/l, respectively (Figure 15.2).

By ensuring residual values of ammonical-nitrogen and orthophosphate-phosphorus in the digester effluent, nitrogen and phosphorus should not be limited in the digester. Residual values of 5 mg/l of NH_4^+–N and 1–2 mg/l of HPO_4^-–P are commonly recommended.

Adequate nutrient needs for anaerobic digesters may be determined by ensuring at least a minimum amount of a nutrient as a percentage of the COD loading to the digester. Table 15.2 lists some nutrient needs.

If nutrient addition is required for nitrogen or phosphorus, several chemicals may be used. For nitrogen addition, ammonium chloride, aqueous ammonia, and urea

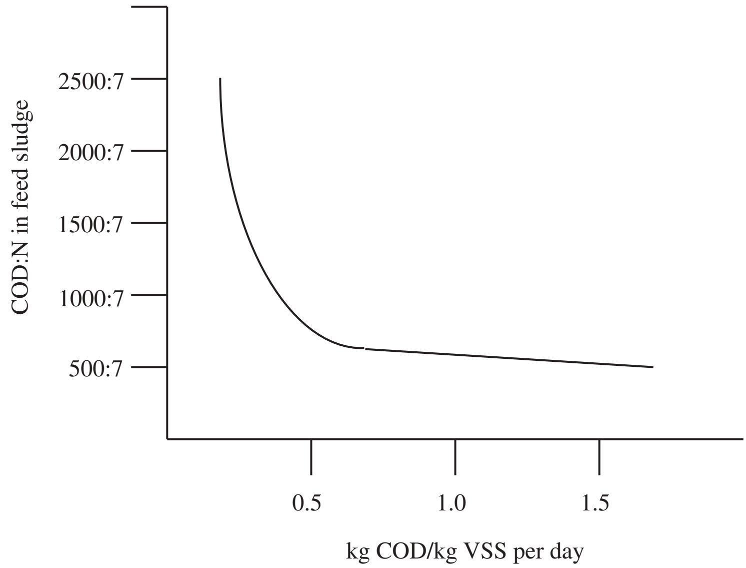


Figure 15.1 *Nutrient needs of an anaerobic digester are determined by the loading or the COD:N and COD:P in the feed sludge. With increasing COD loading there is a corresponding increase in nutrient needs for nitrogen and phosphorus.*

Influent COD	=	10,000 mg/l
Treatment efficiency	=	80%
COD removed	=	8,000 mg/l
Biomass growth (0.1 X 8,000)	=	800 mg VSS/l
Nitrogen required (0.12 X 800)	=	96 mg/l
Phosphorus required (0.02 X 800)	=	16 mg/l

Figure 15.2

TABLE 15.1 Elementary Composition of Bacterial Cells (Dry Weight)

Element	Approximate Percent Composition
Carbon	50
Oxygen	20
Nitrogen	12
Hydrogen	8
Phosphorus	2
Sulfur	1
Potassium	1
Others	6

TABLE 15.2 Significant Nutrient Needs for Anaerobic Digesters

Nutrient	Micronutrient	Macronutrient	Minimum Recommended (% of COD)
Cobalt	X		0.01
Iron	X		0.2
Nickel	X		0.001
Nitrogen		X	3–4
Phosphorus		X	0.5–1
Sulfur	X		0.2

may be used. For phosphorus addition, phosphate salts and phosphoric acid may be used.

MICRONUTRIENTS

Because methane-forming bacteria possess several unique enzyme systems, they have micronutrient requirements that are different from those of other bacteria. The need for several micronutrients, especially cobalt, iron, nickel, and sulfide, is critical. Additional trace elements on which enzymes of methane-forming bacteria are dependent include selenium and tungsten. The incorporation of micronutrients in enzyme systems is essential to ensure not only proper degradation of substrate but also efficient operation of the digester. Cobalt, iron, nickel, and sulfide are obligatory micronutrients, because they are required by methane-forming bacteria to convert acetate to methane. Therefore, attention to macronutrient needs alone is grossly inadequate for methane-forming bacteria.

Molybdenum, tungsten, and selenium may be obligatory micronutrients. Additional micronutrients of concern are barium, calcium, magnesium, and sodium. Deficiencies for micronutrients in anaerobic digesters often have been mistaken for symptoms of toxicity.

Although these micronutrients are usually present in sufficient quantities in municipal wastewater, the digester effluent should be analyzed to ensure that residual soluble quantities of these nutrients exist, especially in industrial wastewater treatment plants. The presence of adequate nutrients, especially micronutrients, helps to minimize digester upsets caused by the accumulation of volatile fatty acids.

Methane-forming bacteria are able to easily remove or "harvest" micronutrients from the bulk solution. The harvesting of micronutrients is accomplished through the production and excretion of extracellular "slime" that chelates and transports the nutrients into the cell (Figure 15.3). The use of extracellular slime permits "luxury" uptake of micronutrients, that is, the removal and storage of nutrients beyond the quantity that is needed.

If it is necessary to add micronutrients to an anaerobic digester, yeast extract can be used. Yeast extract contains numerous amino acids, minerals, and vitamins, including the B vitamins biotin and folic acid. The addition of 1.5 kg/m^3 of yeast extract at all loading rates should provide adequate micronutrients.

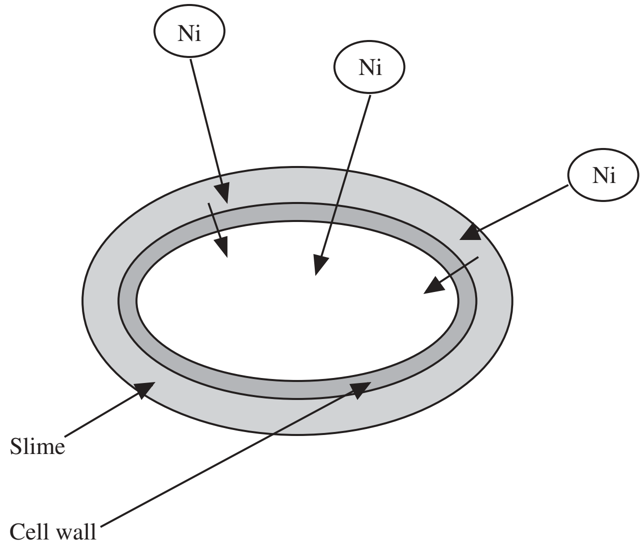


Figure 15.3 Relatively large quantities of micronutrients are removed from the bulk solution by methane-forming bacteria through their adsorption to the slime that coats the bacterial cells. Once adsorbed the nutrients are then absorbed by the bacterial cells.

COBALT

Cobalt is required as an activator of enzyme systems in methane-forming bacteria. The incorporation of cobalt into enzyme systems provides for more efficient conversion of acetate to methane.

IRON

Although methane-forming bacteria have a relatively high iron requirement, and iron usually exists in high concentrations in the environment, it is difficult for methane-forming bacteria as well as anaerobic bacteria in general to assimilate iron. For iron to be assimilated, it must be in solution. Unfortunately, this requirement is usually not satisfied in the environment of methane-forming bacteria and other anaerobic bacteria.

NICKEL

Nickel is a unique micronutrient requirement for methane-forming bacteria, because nickel is generally not essential for the growth of most bacteria. For example, the F_{430} enzyme in methane-forming bacteria contains nickel. The addition of nickel can increase acetate utilization rate of methane-forming bacteria.

The requirement for nickel has long been overlooked because of the high background level or presence of nickel in bacterial growth media. However, the lack of adequate usable nickel in the bulk solution of an anaerobic digester results in a

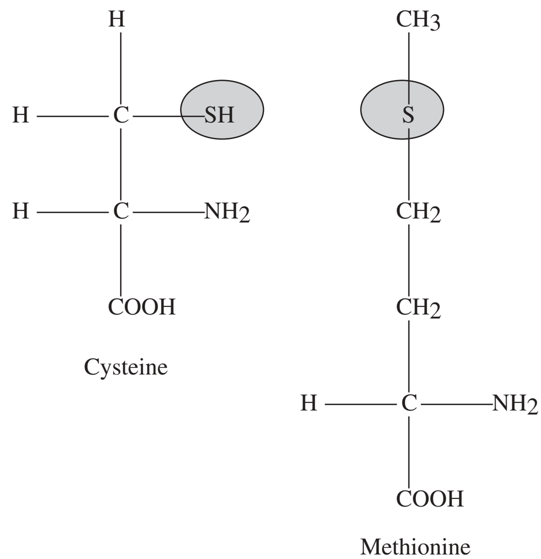


Figure 15.4

significant decrease in the rate of methane production, that is, decreased enzymatic ability to convert acetate to methane.

SULFIDE

Sulfide is the principle source of sulfur for methane-forming bacteria. For sulfide to enter a bacterial cell, it must exist as nonionized hydrogen sulfide (H_2S). This form of sulfide occurs in a relatively high concentration within the pH range of 6.8 to 6.9, which is also near the pH of normal anaerobic digester operation.

Additional sulfur sources for methane-forming bacteria are the amino acids cysteine and methionine (Figure 15.4). These amino acids contain sulfur (S) or the thiol group (–SH), which releases sulfur on degradation of the amino acids.

Although sulfide is considered a micronutrient for methane-forming bacteria, the sulfide content of these bacteria is relatively high. On a dry weight basis, approximately 2.5% of the bacterial cell is sulfide. This quantity of sulfide also is approximately 50% greater than the phosphorus content of the cell.

Although sulfide is required in relatively high concentrations and is considered an obligate micronutrient, relatively high concentrations of sulfide present two significant problems for successful anaerobic digester operation. Sulfide presents operational problems by precipitating trace metals or micronutrients and causing toxicity at high concentrations.

16

Alkalinity and pH

Sufficient alkalinity is essential for proper pH control. Alkalinity serves as a buffer that prevents rapid change in pH. Enzymatic activity or digester performance is influenced by pH. Acceptable enzymatic activity of acid-forming bacteria occurs above pH 5.0, but acceptable enzymatic activity of methane-forming bacteria does not occur below pH 6.2. Most anaerobic bacteria, including methane-forming bacteria, perform well within a pH range of 6.8 to 7.2.

The pH in an anaerobic digester initially will decrease with the production of volatile acids. However, as methane-forming bacteria consume the volatile acids and alkalinity is produced, the pH of the digester increases and then stabilizes. At hydraulic retention times >5 days, the methane-forming bacteria begin to rapidly consume the volatile acids.

In a properly operating anaerobic digester a pH of between 6.8 and 7.2 occurs as volatile acids are converted to methane and carbon dioxide (CO_2). The pH of an anaerobic system is significantly affected by the carbon dioxide content of the biogas.

Digester stability is enhanced by a high alkalinity concentration. A decrease in alkalinity below the normal operating level has been used as an indicator of pending failure. A decrease in alkalinity can be caused by 1) an accumulation of organic acids due to the failure of methane-forming bacteria to convert the organic acids to methane, 2) a slug discharge of organic acids to the anaerobic digester, or 3) the presence of wastes that inhibit the activity of methane-forming bacteria. A decrease in alkalinity usually precedes a rapid change in pH.

The composition and concentration of the feed sludge directly influence the alkalinity of the digester. For example, large quantities of proteinaceous wastes transferred to the anaerobic digester are associated with relatively high concentrations

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of alkalinity. The alkalinity is the result of the release of amino groups ($-NH_2$) and production of ammonia (NH_3) as the proteinaceous wastes are degraded. Also, thickened sludges have relatively high alkalinity. This alkalinity is due to the increased feed rate of proteins within the thickened sludges.

Alkalinity is present primarily in the form of bicarbonates that are in equilibrium with carbon dioxide in the biogas at a given pH. When organic compounds are degraded, carbon dioxide is released. When amino acids and proteins are degraded, carbon dioxide and ammonia are released.

The release of carbon dioxide results in the production of carbonic acid, bicarbonate alkalinity, and carbonate alkalinity (Equation 16.1). The release of ammonia results in the production of ammonium ions (Equation 16.2).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (16.1)$$

$$NH_3 + H^+ \leftrightarrow NH_4^+ \quad (16.2)$$

The equilibrium between carbonic acid, bicarbonate alkalinity, and carbonate alkalinity as well as ammonia and ammonium ions is a function of digester pH (Figure 16.1). Bicarbonate alkalinity is the primary source of carbon for methane-forming bacteria.

Significant changes in alkalinity or pH are introduced in an anaerobic digester by substrate feed or the production of acidic and alkali compounds, such as organic acids and ammonium ions, respectively, during the degradation of organic compounds in the digester.

Alkalinity in an anaerobic digester also is derived from the degradation of organic-nitrogen compounds, such as amino acids and proteins, and the production of carbon dioxide from the degradation of organic compounds. When amino acids and proteins are degraded, amino groups ($-NH_2$) are released and alkalinity is produced. When amino groups are released, ammonia is produced. The ammonia

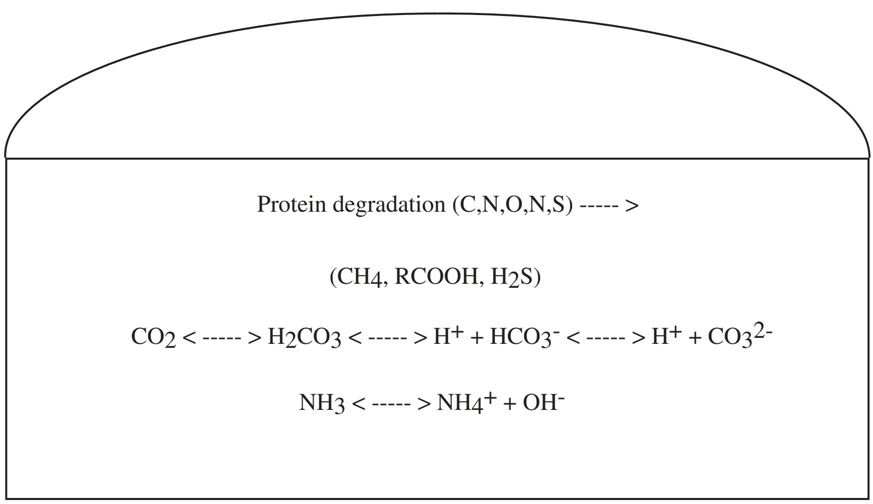


Figure 16.1

dissolves in water along with carbon dioxide to form ammonium bicarbonate (NH_4HCO_3) (Equation 16.3).

$$NH_3 + H_2O + CO_2 \leftrightarrow NH_4HCO_3 \tag{16.3}$$

However, the degradation of organic compounds produces organic acids that destroy alkalinity. For example, as a result of the degradation of glucose, acetate is formed (Equation 16.4). This acid destroys alkalinity, for example, ammonium bicarbonate (Equation 16.5), and the alkalinity is not returned until methane fermentation occurs (Equation 16.6).

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \tag{16.4}$$

$$3CH_3COOH + 3NH_4HCO_3 \rightarrow 3CH_4COONH_4 + 3H_2O + 3CO_2 \tag{16.5}$$

$$3CH_3COONH_4^+ + 3H_2O \rightarrow 3CH_4 + 3NH_4HCO_3 \tag{16.6}$$

Although anaerobic digester efficiency is satisfactory within the pH range of 6.8 to 7.2, it is best when the pH is within the range of 7.0 to 7.2. Values of pH below 6 or above 8 are restrictive and somewhat toxic to methane-forming bacteria (Table 16.1). To maintain a stable pH, a high level of alkalinity is required.

If the feed sludge to the anaerobic digester does not contain alkali compounds or precursors of alkali compounds, alkalinity must be added to the digester to maintain stable and acceptable values for alkalinity and pH. The quantity of alkalinity to be added should be based on the anticipated organic acid production capacity of the sludge feed (1 g of volatile acids per gram of volatile solids). Also, if the rate of acid production exceeds the rate of methane production, alkalinity must be added. A higher rate of volatile acid production than methane production usually occurs during start-up, overload, loss of adequate temperature, and inhibition.

Alkalinity also may be lost or "washed out" of the digester. When increased wastewater temperature occurs, increased microbial activity within an activated sludge process occurs and buoyant sludge is usually produced. Increased pumping from the activated sludge process or thickener to the anaerobic digester occurs because of the presence of buoyant sludge. Increased pumping produces decreased digester hydraulic retention time (HRT) and "washout" of digester alkalinity.

TABLE 16.1 Optimum Growth pH of Some Methane-forming Bacteria

Genus	pH
Methanosphaera	6.8
Methanothermus	6.5
Methanogenium	7.0
Methanolacinia	6.6–7.2
Methanomicrobium	6.1–6.9
Methanospirillium	7.0–7.5
Methanococcoides	7.0–7.5
Methanohalobium	6.5–7.5
Methanolobus	6.5–6.8
Methanothrix	7.1–7.8

TABLE 16.2 Chemicals Commonly Used for Alkalinity Addition

Chemical	Formula	Buffering Cation
Sodium bicarbonate	$NaHCO_3$	Na^+
Potassium bicarbonate	$KHCO_3$	K^+
Sodium carbonate (soda ash)	Na_2CO_3	Na^+
Potassium carbonate	K_2CO_3	K^+
Calcium carbonate (lime)	$CaCO_3$	Ca^{2+}
Calcium hydroxide (quick lime)	$Ca(OH)_2$	Ca^{2+}
Anhydrous ammonia (gas)	NH_3	NH^{4+}
Sodium nitrate	$NaNO_3$	Na^+

Several chemicals can be used to adjust alkalinity and pH in an anaerobic digester (Table 16.2). Because methane-forming bacteria require bicarbonate alkalinity, chemicals that release bicarbonate alkalinity directly are preferred. Of these chemicals, sodium bicarbonate and potassium bicarbonate are perhaps the best chemicals of choice because of their desirable solubility, handling, and minimal adverse impacts within the digester. For example, overdosing of these chemicals does not cause the pH of the digester to quickly rise above the optimum. Also, of all the cations released by the alkali chemicals used for alkalinity addition, sodium and potassium are the least toxic to the bacteria in the digester. Chemicals that release hydroxide alkalinity, for example, caustic soda, are not effective in maintaining proper alkalinity in the digester because of the bicarbonate alkalinity requirement of methane-forming bacteria.

Lime ($CaCO_3$) may be used to increase digester pH to 6.4, and then either bicarbonate or carbonate salts (sodium or potassium) should be used to increase the pH to the optimum range. Lime increases pH quickly and dramatically, but lime does not significantly increase alkalinity. Overdosing with lime may easily cause the pH to exceed the optimum pH range.

Caution should be used when using hydrated lime or quick lime [calcium hydroxide ($Ca(OH)_2$)] and soda ash [sodium carbonate (Na_2CO_3)] to increase alkalinity. Calcium hydroxide and sodium carbonate first react with soluble carbon dioxide in the sludge (Equations 16.7 and 16.8, respectively). If carbon dioxide is removed too rapidly or in too large a quantity from the sludge, then carbon dioxide from the biogas will replace the carbon dioxide lost from the sludge. When carbon dioxide is lost from the biogas, a partial vacuum condition develops under the digester dome. This condition may cause the digester cover to collapse. Also, as the concentration of alkalinity increases in the anaerobic digester, the continued use of quick lime results in the precipitation of calcium carbonate (Equation 16.9).

$$Ca(OH)_2 + 2CO_2 \rightarrow Ca(HCO_3)_2 \quad (16.7)$$

$$Na_2CO_3 + H_2O + CO_2 \rightarrow 2NaHCO_3 \quad (16.8)$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \quad (16.9)$$

Anhydrous ammonia also may be used to adjust alkalinity and pH. Ammonia reacts with carbon dioxide and water, resulting in the production of ammonium

bicarbonate (Equation 16.10). Ammonium carbonate adds alkalinity and is available to react with volatile acids, resulting in the production of volatile acid salts (Equation 16.11).

$$NH_3 + CO_2 + H2O \rightarrow NH_4HCO_3 \quad (16.10)$$

$$NH_4HCO_3 + RCOOH^* \rightarrow RCOONH^4 + H^+ + HCO_3^- \quad (16.11)$$

*R represents the non-carboxyl (–COOH) portion of the volatile acid.

Anhydrous ammonia also may help to dissolve scum layers. Although the addition of anhydrous ammonia has several benefits for an anaerobic digester, there are some concerns. Anhydrous ammonia may produce a negative pressure in the digester by reacting with carbon dioxide. In addition, at elevated pH values excess ammonia gas may cause toxicity.

If pH and alkalinity both must be increased in an anaerobic digester, sodium carbonate may be used to increase pH if it drops below 6.5. Sodium carbonate also replenishes alkalinity. If sodium bicarbonate, sodium carbonate, or sodium nitrate is added too rapidly to an anaerobic digester, a foaming problem may develop. Sodium bicarbonate and sodium carbonate release carbon dioxide on addition, whereas sodium nitrate releases molecular nitrogen (N_2) and nitrous oxide (N_2O) upon addition.

Caution also should be used when adding sodium nitrate, because the release of nitrate ions (NO_3^-) increases the oxidation-reduction potential (ORP) of the digester. The ORP of the digester should not be allowed to increase above –300 mV, for example, –250 mV, because methane-forming bacteria cannot produce methane at ORP values greater than –300 mV in a mixed culture.

Any chemical selected for addition to the digester should be added slowly to prevent any adverse impact on the bacteria due to rapid changes in alkalinity, pH, ionic strength, or ORP.

Caution should be exercised in the choice of the chemical used for pH/alkalinity adjustments. The precipitation of $CaCO_3$ creates unwanted solids, and the large quantities of a single cation, for example, Na^+, presents the potential for alkali metal toxicity. Therefore, it may be preferable to use mixtures of cations, for example, Ca^{2+} from $Ca(OH)_2$, Na^+ from NaOH, and K^+ from KOH, for pH/alkalinity control.

Although the pH of the digester is more easily and quickly determined than the alkalinity of the digester, the pH is only an indication of what has already happened in the digester, whereas changes in alkalinity indicate what is happening in the digester. The alkalinity of the digester indicates whether alkalinity addition or corrective measures are needed.

Excessive alkalinity in the digester should be avoided. Excess alkalinity can be destroyed or neutralized with the addition of ferric chloride or citrate.

17

Toxicity

A variety of inorganic and organic wastes can cause toxicity in anaerobic digesters (Table 17.1). Many toxic wastes are removed in primary clarifiers and transferred directly to the anaerobic digester. Heavy metals may be precipitated as hydroxides in primary sludge, and organic compounds such as oils and chloroform are removed in primary scum and sludge, respectively. Industrial wastewaters often contain wastes that are toxic to anaerobic digesters.

Although guideline values or ranges of values exist at which toxicity occurs for specific inorganic wastes (Table 17.2) and organic wastes (Table 17.3), methane-bacteria often can tolerate higher values by acclimating to the wastes. When toxic values of specific wastes for anaerobic digesters are assessed, the toxic value is determined by several factors. These factors include 1) the ability of the bacteria to adapt to a constant concentration of toxic waste, 2) the absence or presence of other toxic wastes, and 3) changes in operational conditions.

Toxicity in an anaerobic digester may be acute or chronic. Acute toxicity results from the rapid exposure of an unacclimated population of bacteria to a relatively high concentration of a toxic waste. Chronic toxicity results from the gradual and relatively long exposure of an unacclimated population of bacteria to a toxic waste.

The population of bacteria may acclimate under chronic toxicity by two means. First, they may repair damaged enzyme systems in order to adjust to the toxic wastes or degrade the toxic organic compound. Second, they may grow a relatively large population of bacteria that is capable of developing the enzyme systems necessary to degrade the toxic organic compounds. The time of chronic toxicity in an anaerobic digester is determined by 1) the time of contact between the toxic waste and the bacteria and 2) the ratio of toxic waste to the bacterial population (biomass or solids).

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TABLE 17.1 Inorganic and Organic Toxic Wastes to Anaerobic Digesters

Alcohols (isopropanol)
Alkaline cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+})
Alternate electron acceptors, nitrate (NO_3^-) and sulfate (SO_4^{2-})
Ammonia
Benzene ring compounds
Cell bursting agent (lauryl sulfate)
Chemical inhibitors used as food preservatives
Chlorinated hydrocarbons
Cyanide
Detergents and disinfectants
Feedback inhibition
Food preservatives
Formaldehyde
Heavy metals
Hydrogen sulfide
Organic-nitrogen compounds (acrylonitrile)
Oxygen
Pharmaceuticals (monensin)
Solvents
Volatile acids and long-chain fatty acids

TABLE 17.2 Toxic Values for Selected Inorganic Wastes

Waste	Concentration (mg/l) in Influent to Digester
Ammonia	1500
Arsenic	1.6
Boron	2
Cadmium	0.02
Chromium (Cr^{6+})	5–50
Chromium (Cr^{3+})	50–500
Copper	1–10
Cyanide	4
Iron	5
Magnesium	1000
Sodium	3500
Sulfide	50
Zinc	5–20

TABLE 17.3 Toxic Values for Selected Organic Wastes

Waste	Concentration (mg/l) in influent to digester
Alcohol, allyl	100
Alcohol, octyl	200
Acrylonitrile	5
Benzidine	5
Chloroform	10–16
Carbon tetrachloride	10–20
Methylene chloride	100–500
1,1,1-Trichloroethane	1
Trichlorofluoromethane	20
Trichlorotrifluoroethane	5

Indicators of toxicity in an anaerobic digester may appear rapidly or slowly depending on the type of toxicity and the concentration of the toxic waste. Indicators of toxicity include the disappearance of hydrogen, the disappearance of methane, decreases in alkalinity and pH, and an increase in volatile acid concentration.

Wastes that are toxic to anaerobic digesters are numerous and diverse. Perhaps the three most commonly reviewed types of toxicity are ammonia, hydrogen sulfide, and heavy metals. Additional types of toxic wastes are listed in Table 17.1 and may be found in simple household detergents and complex anthropogenic organic compounds. Household detergents that contain the dispersing agent lauryl sulfate burst the cell walls of bacteria. Anthropogenic organic compounds include solvents and pesticides. These compounds are either highly chlorinated or contain cyanide (CN).

AMMONIA TOXICITY

Ammonical-nitrogen (NH_4^+–N) or ammonium ions (NH_4^+), a reduced form of nitrogen, may be transferred to an anaerobic digester or may be produced during the anaerobic degradation of organic nitrogen compounds such as amino acids and proteins. Reduced nitrogen exits in two forms, the ammonium ion and free or nonionized ammonia (NH_3). The effects of ammonical-nitrogen/ammonia in the anaerobic digester are positive and negative (Table 17.4). Ammonium ions are used by bacteria in the anaerobic digester as a nutrient source for nitrogen. Free ammonia is toxic.

The amount of each form of reduced nitrogen in an anaerobic digester is determined by the digester pH, and the forms are in relatively equal amounts at pH 9.3 (Equation 17.1). With increasing pH, the amount of free ammonia increases. With decreasing pH, the amount of ammonium ions increases. At pH 7, free ammonia accounts for approximately 0.5% of the total reduced nitrogen.

$$NH_4^+ \leftrightarrow NH_3 + H^+ \tag{17.1}$$

Free ammonia is toxic to methane-forming bacteria. The toxic effects of ammonia as well as cyanide and hydrogen sulfide are determined by digester pH. All are toxic in their undissociated (nonionized) form, that is, NH_3, HCN (Equation 17.2), and H_2S (Equation 17.3). The pH effect on ammonia is direct, that is, with increasing pH ammonia is produced in large quantities. The pH effect on cyanide and hydrogen sulfide is indirect, that is, with decreasing pH cyanide and hydrogen sulfide are produced in large quantities. Although methane-forming bacteria can acclimate to free ammonia, unacclimated methane-forming bacteria can be inhibited at free ammonia concentrations >50 mg/l.

TABLE 17.4 Effects of Ammonical-nitrogen/Ammonia in an Anaerobic Digester

Ammonical-nitrogen (NH_4^+)/Dissolved Ammonia (NH_3), N	Effect
50–200 mg/l	Beneficial
200–1000 mg/l	No adverse effect
1500–3000 mg/l	Inhibitory at pH > 7

$$HCN \leftrightarrow CN^- + H^+ \tag{17.2}$$

$$H_2S \leftrightarrow HS^- + H^+ \tag{17.3}$$

Concentrations of ammonia >50 mg/l can be tolerated by methane-forming bacteria if the bacteria have been acclimated. If methane-forming bacteria cannot be acclimated to free ammonia, digester pH can be decreased or digester feed sludge can be diluted to prevent ammonia toxicity.

The toxic effects of free ammonia may be confined to methane-forming bacteria, and the precise concentration at which free ammonia is toxic remains uncertain. However, anaerobic digesters with acclimated populations of methane-forming bacteria can tolerate several hundred milligrams per liter of free ammonia. Ammonia concentrations >1500 mg/l at high pH may result in digester failure. At concentrations above 3000 mg/l, free ammonia becomes toxic enough to cause digester failure.

Variations in concentrations of free ammonia toxicity result from several operational factors. These factors include digester alkalinity or buffering capacity, temperature, and sludge loading rates.

Although relatively high concentrations of free ammonia, for example, 1500–3000 mg/l, can be inhibitory to methane-forming bacteria, ammonia inhibition may be "self-correcting." Because methane-forming bacteria are inhibited by free ammonia, volatile acid concentration increases. With an increase in digester volatile acids, the pH of the digester drops. The drop in pH converts much of the free ammonia to ammonium ions.

A shock load of free ammonia (a concentration greater than the digester design limit) causes a rapid and large accumulation of volatile acids and a rapid and significant drop in pH. Besides volatile acid accumulation, loss of alkalinity, and drop in pH, a decrease in methane production also is indicative of ammonia toxicity.

Ammonium ions perform several important roles in an anaerobic digester. Ammonium ions are the preferred bacterial nutrient for nitrogen. They also provide buffering capacity in an anaerobic digester. However, although ammonium bicarbonate acts as a buffer, high ammonium bicarbonate concentrations resulting from the degradation of amino acids, proteins, and highly concentrated sludges may cause free ammonia toxicity.

A common cause of digester failure is the presence of an unacclimated population of methane-forming bacteria at high ammonia concentrations. Therefore, methane-forming bacteria should be gradually acclimated to increasing concentrations of ammonia.

HYDROGEN SULFIDE

Bacterial cells need soluble sulfur as a growth nutrient and satisfy this need by using soluble sulfide (HS^-). However, excessive concentrations of sulfides or dissolved hydrogen sulfide gas (H_2S) cause toxicity.

Hydrogen sulfide is one of the compounds most toxic to anaerobic digesters. The methane-forming bacteria are the bacteria that are most susceptible to hydrogen sulfide toxicity. Hydrogen-consuming methane-forming bacteria are more susceptible to hydrogen sulfide toxicity than acetoclastic methane-forming bacteria. Acid-forming bacteria also are susceptible to hydrogen sulfide toxicity.

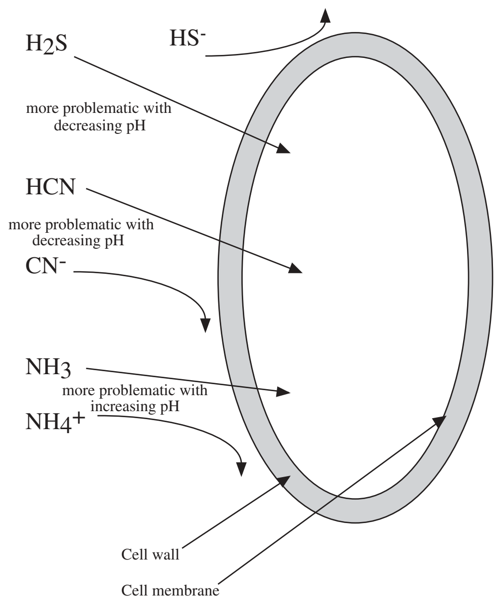


Figure 17.1 *The toxicity of hydrogen sulfide, hydrogen cyanide, and ammonia are pH dependent. In the non-ionized forms (H_2S, HCN, and NH_3) toxicity can occur. In the non-ionized forms these molecules are capable of easily entering the bacterial cell and attacking enzyme systems.*

Soluble hydrogen sulfide toxicity occurs because sulfide inhibits the metabolic activity of anaerobic bacteria. Although the mechanism by which sulfide inhibits anaerobic bacteria is not completely understood, toxicity can occur at concentrations as low as 200 mg/l at neutral pH. Because diffusion through a cell membrane is required to exert toxicity and non-ionized hydrogen sulfide diffuses more rapidly across a cell membrane than sulfide, hydrogen sulfide toxicity is pH dependent (Figure 17.1).

Hydrogen sulfide is formed in anaerobic digesters from the reduction of sulfate and the degradation of organic compounds such as sulfur-containing amino acids and proteins. The amino acids cystine, cysteine, and methionine that are incorporated into many proteins contain sulfur in a thiol group (–SH) that is released during the degradation of the amino acids (Figure 17.2).

Sulfate is relatively non-inhibitory to methane-forming bacteria. Sulfate is reduced to hydrogen sulfide by sulfate-reducing bacteria (SRB). For each gram of chemical oxygen demand (COD) degraded by SRB 1.5 grams of sulfate are reduced to hydrogen sulfide.

Several genera of anaerobic bacteria reduce sulfate or sulfur to hydrogen sulfide. The genus name of these bacteria begins with the prefix "*Desulf*." The genera include *Desulfuromonas*, *Desulfovibrio*, and *Desulfomonas*. SRB are similar to methane-forming bacteria with respect to habitat and cellular morphology or structure.

The presence of hydrogen sulfide also can be due to the reduction of elemental sulfur. An additional source of sulfides is sulfate salts present in wastewaters from metallurgical industries.

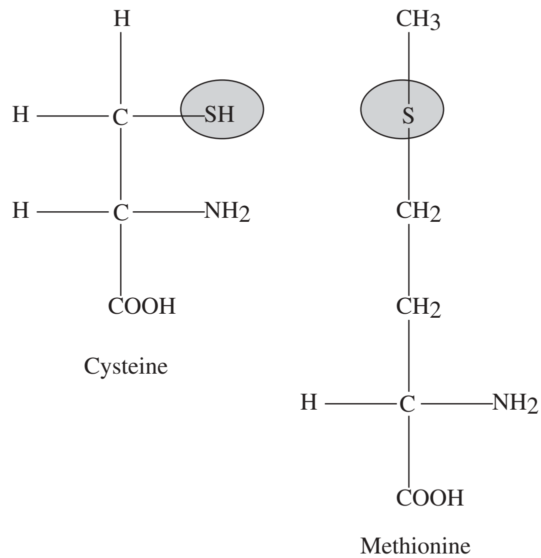


Figure 17.2

Sulfide in an anaerobic digester may be in the soluble or insoluble form. In the insoluble form such as lead sulfide (PbS) and iron sulfide (Fe_2S_3), sulfide does not exert toxicity. Insoluble sulfide cannot enter bacterial cells. A common operational practice to prevent sulfide toxicity in anaerobic digesters is to add iron. This practice precipitates the sulfide as iron sulfide, which gives the treated sludge a black color. Dissolved sulfide can react with any heavy metal except chromium.

Although some of the sulfide leaves the digester sludge as free hydrogen sulfide gas, and some is precipitated as heavy metal salts, a portion of the sulfide remains dissolved. Concentrations of dissolved hydrogen sulfide above 200 mg/l are toxic and should be reduced.

Free hydrogen sulfide gas can be removed from digester sludge by the rapid production of carbon dioxide, hydrogen, and methane. Treatment measures that can be used to reduce soluble hydrogen sulfide include 1) diluting the sulfides, 2) separating and treating the sulfate/sulfide waste stream, 3) precipitating the sulfide as a metal salt, and 4) scrubbing and recirculating digester biogas.

Sulfide toxicity is most likely to occur under low organic loadings. Under these conditions, insufficient biogas is produced. This deficiency in biogas production results in poor stripping of sulfide from the sludge.

HEAVY METALS

Numerous heavy metals such as cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) are found in wastewaters and sludges and are transferred to anaerobic digesters. These metals are referred to as "heavy" because of their undesired impact on wastewater treatment processes and operational costs including their accumulation in sludges. High concentrations of metals in sludges affect sludge disposal options and costs.

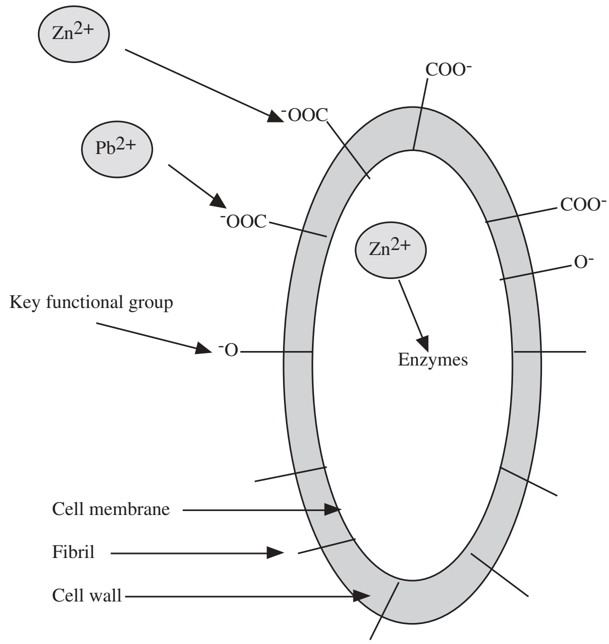


Figure 17.3 *Heavy metals cause toxicity in the soluble form. The metals are adsorbed to the surface of the negatively charged, bacterial fibrils that extend into the bulk solution from the cell membrane through the cell wall. The fibrils are negatively charged by the ionization (loss of hydrogen) from key functional groups such as carboxyl (–COOH) and hydroxyl (–OH). Once adsorbed the metals are then absorbed by the bacterial cells. Inside the cells the metals attack enzyme systems.*

Although some heavy metals (cobalt, molybdenum, and nickel) at trace concentrations serve as additives or activators that enhance enzymatic activity of methane-forming bacteria, heavy metals in moderate to excessive concentrations may cause toxicity in anaerobic digesters.

Soluble heavy metals are removed from wastewaters and sludges through their adsorption to the surface of bacterial cells (Figure 17.3). Once absorbed, heavy metals exert toxicity by inactivating enzymatic systems. Inactivation occurs when the metals bind to the thiol groups in enzymes. Inactivation of enzymes results in digester failure. The concentration at which heavy metals exert toxicity is dependent on the composition of the digester feed sludge.

Although heavy metals often are present in relatively high concentrations in anaerobic digesters, these metals usually do not cause toxicity. Most heavy metals are combined—not free—therefore, they cannot be adsorbed or absorbed by bacteria, and toxicity cannot occur.

Heavy metals can be combined through several mechanisms. Metal ions may be bonded to a variety of naturally occurring chelating compounds that are found in domestic and municipal wastewaters. Chelated metals cannot enter bacterial cells. Many metals in anaerobic digesters are present in the form of insoluble salts or precipitates of oxides, hydroxides, sulfides, and carbonates. At pH values >7.5 significant precipitation of the salts of carbonate and sulfides occurs. Precipitated metals cannot enter bacterial cells. Metal salts in the form of chlorides and nitrates are soluble and undergo ionization that releases soluble heavy metal ions.

Heavy metal ions that are very toxic to methane-forming bacteria at relatively low concentrations are copper, nickel, and zinc. These ions are soluble in anaerobic digesters. Reacting the ions to precipitate as metal sulfides can reduce the toxicity of these ions. Approximately 2 mg/l of ions are precipitated as metal sulfides by 1 mg/l of sulfide.

ALTERNATE ELECTRON ACCEPTORS

The presence of nitrate ions (NO_3^-) or sulfate ions (SO_4^{2-}) may inhibit methane-forming bacteria. Nitrate ions and sulfate ions may be found in relatively high concentrations in industrial wastewaters or aerated and nitrified municipal wastewaters and sludges.

Both ions adversely impact the activity of methane-forming bacteria by increasing the redox value within the anaerobic digester. Low redox values (less than –300 mV) are required for proper activity of methane-forming bacteria.

Because SRB can out-compete methane-forming bacteria for substrates (acetate, alcohols, formate, hydrogen, and carbon dioxide) that are used for methane production, hydrogen sulfide production predominates over methane production. Here, organic compounds are oxidized to carbon dioxide and sulfate is reduced to hydrogen sulfide.

ALKALINE CATIONS

Four cations are associated with alkali compounds. These cations or metals are calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na). The salts of these metals, for example, sodium hydroxide (NaOH), often are added to anaerobic digesters to increase alkalinity and pH. The cations also may be transferred to anaerobic digesters from industrial wastes.

The cations have stimulatory and inhibitory effects on anaerobic digesters. At relatively low concentrations (100–400 mg/l) the cations are desirable and enhance anaerobic bacterial activity. At concentrations >1500 mg/l the cations begin to exhibit significant toxicity. Diluting the cation concentration can prevent cation toxicity.

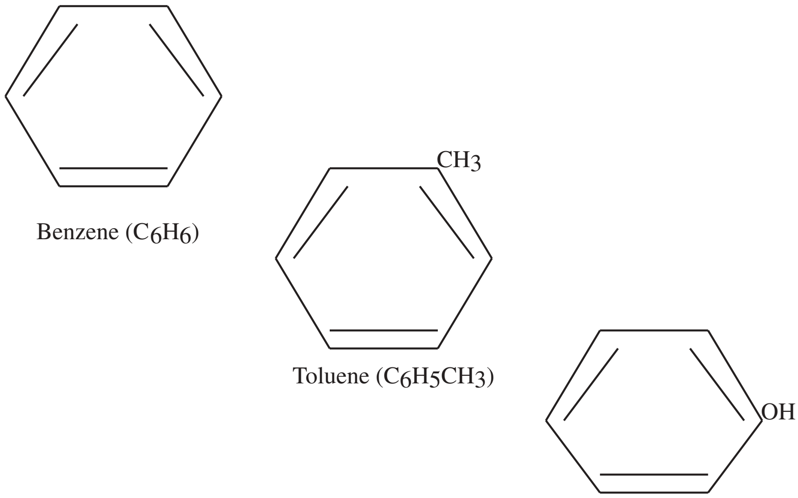
BENZENE RING COMPOUNDS

Methane-forming bacteria are inhibited by a variety of benzene ring compounds (Figure 17.4). These compounds include benzene, pentachlorophenol, phenol, phenolic compounds, and toluene.

Phenolic compounds include chlorophenols, nitrophenols, and tannins. Tannins are naturally occurring phenolic compounds found in fruits and vegetables, for example, apples, bananas, beans, cereals, and coffee. Tannins may exert toxicity at 700 mg/l.

CHLORINATED HYDROCARBONS

Chlorinated hydrocarbons are toxic to methane-forming bacteria (Table 17.4). Chloroform, for example, is toxic at a concentrations of 15 mg/l. However, methane-forming bacteria can acclimated to many chlorinated hydrocarbons.



Benzene (C_6H_6)

Toluene ($C_6H_5CH_3$)

Phenol (C_6H_5OH)

Figure 17.4

CYANIDE

Cyanide (–CN) and cyanide-containing compounds (cyano-compounds) are commonly found in industrial wastewaters from metal cleaning and electroplating firms. In the metal finishing industry they are used in plating baths. Cyanide and cyano-compounds are toxic to methane-forming bacteria. Toxicity occurs at cyanide concentrations >100 mg/l. Cyanide prevents methane production from acetate, but it may not prevent methane production from carbon dioxide and methanol. However, cyanide toxicity is reversible. The reversibility of toxicity is dependent on the concentration of cyanide and its time in the digester as well as the concentration of solids (bacteria) in the digester, solids retention time (SRT), and temperature.

FEEDBACK INHIBITION

Fermentation often results in the production of several intermediates such as hydrogen and volatile fatty acids that are toxic. The presence of toxicity that is caused by the production of hydrogen and volatile fatty acids is referred to as feedback inhibition.

Excess hydrogen production and accumulation results in increased partial hydrogen pressure. This increased pressure inhibits acetate-forming bacteria. Excess volatile fatty acid production and accumulation inhibits methane-forming bacteria through direct toxicity such as that caused by propionate or decreased alkalinity and pH.

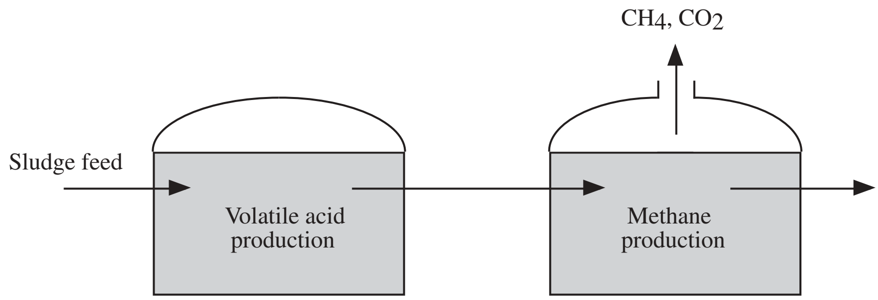


Figure 17.5

TABLE 17.5 Chlorinated Hydrocarbons that are Toxic to Methane-forming Bacteria

Chloroform
Hexachlorocyclopentadiene
Hexachloroethane
Hexachloro-1,3-butadiene
2,4-Dichlorophenol

Feedback inhibition may be overcome by using a two-phase anaerobic digester system (Figure 17.5). This system separates volatile acid production and methane production. The system also provides improved stability and increased resistance to toxic wastes. Long SRTs also allow the bacteria to increase in number and permit the bacteria to acclimate to toxic wastes.

FORMALDEHYDE AND PHENOLIC WASTES

Formaldehyde (H_2CO) is an example of an organic compound that is degradable at low concentrations but toxic at high concentrations. Phenolic wastes are additional examples (Table 17.5).

Formaldehyde is toxic to methane-forming bacteria. Toxicity occurs at concentrations >100 mg/l. The inhibited activity of methane-forming bacteria recovers at lower concentrations.

VOLATILE ACIDS AND LONG-CHAIN FATTY ACIDS

The presence of a relatively high concentration of short-chain (1–3 carbon units), nonionized volatile acids such as acetate, butyrate, and propionate causes a decrease in the concentration of alkalinity and a drop in pH. Propionate is perhaps the most toxic of the volatile acids and may exert toxicity at concentrations <5 mg/l.

Toxicity is exerted at near-neutral pH values and occurs in populations of acid-forming bacteria and methane-forming bacteria. The presence of an excess concentration of volatile acids can be corrected with the addition of an alkaline compound.

TABLE 17.6 Phenolic Wastes That Are Toxic to Methane-forming Bacteria

Nitrobenzene
2-Nitrophenol
4-Nitrophenol

TABLE 17.7 Long-Chain Fatty Acids That Inhibit Methane Production from Acetate

Fatty Acid	Carbon Units	Saturated/ Unsaturated	Formula
Caprylic (octanoic)	8	Saturated	$CH_3(CH_2)_6COOH$
Capric (decanoic)	10	Saturated	$CH_3(CH_2)_8COOH$
Lauric (dodecanoic)	12	Saturated	$CH_3(CH_2)_{10}COOH$
Myristic (tetradecanoic)	14	Saturated	$CH_3(CH_2)_{12}COOH$
Oleic (*cis*-9-octadecanoic)	18	Unsaturated	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$

Because the chemical composition and structure of several long-chain fatty acids are similar to those of the lipid components in the cell wall of acetoclastic bacteria and methane-forming bacteria, the fatty acids dissolve in the cell wall. Once dissolved in the cell wall, the acids inhibit the activity of the bacteria at very low concentrations.

Long-chain fatty acids of concern include capric, caprylic, lauric, myristic, and oleic acids (Table 17.6). The acids contain carbon chains of 8 to 18 units. Although lauric acid is the most toxic of the long-chain fatty acids, combinations of these acids produce a synergetic effect. Wastewaters that contain significant quantities of long-chain fatty acids include domestic, edible oil refinery, palm oil processing, slaughterhouse, and wool scouring (coning oil). Long-chain fatty acids concentrations >500 g/l may cause toxicity in anaerobic digesters.

RECALCITRANT COMPOUNDS

Difficult to degrade or recalcitrant compounds in anaerobic digesters may cause toxicity to methane-forming bacteria. Examples of these compounds include aliphatic hydrocarbons and some chlorinated compounds such as lignin, humic substances, and chlorinated biphenyls. The recalcitrant compounds become even more difficult to degrade when they contain alkyl groups, halogens, nitro groups, and sulfo groups.

18

Mixing

Anaerobic digester content should be mixed. Mixing enhances the digestion process by distributing bacteria, substrate, and nutrients throughout the digester as well as equalizing temperature. The metabolic activities of acetate-forming bacteria and methane-forming bacteria require that they be in close spatial contact. Slow, gentle mixing ensures that contact. Also, mixing provides for efficient hydrolysis of wastes and production of organic acids and alcohols by acid-forming bacteria. For example, insoluble starches are kept from clumping by mixing action. This allows the hydrolytic bacteria to attack a much larger surface area of the starches and provides for their rapid hydrolysis.

Mixing minimizes the settling of grit and reduces the buildup of scum. Over lengthy periods of operation, solids accumulation can reduce digester performance as the reactor hydraulics become restricted by localized dead volumes and short-circuiting of sludge flow. The advantages of mixing digester content are listed in Table 18.1.

Mixing can be accomplished through mechanical methods or gas recirculation. These methods include external pumps, gas injection or recirculation from the floor or roof of the digester, propellers or turbines, and draft tubes. Mechanical mixers are more effective than gas recirculation, but they often become clogged or fouled with digester solids.

Mixing methods may be grouped into two modes. An intermediate mode incorporates heating with limited mixing achieved through the recycle of sludge in a heat exchanger (Figure 18.1). A rapid mode or high rate (Figure 18.2) incorporates heating and complete mixing and provides significant volatile solids destruction (Figure 18.2).

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TABLE 18.1 Advantages of Mixing Digester Content

Eliminating or reducing scum buildup
Eliminating thermal stratification or localized pockets of depressed temperature
Maintaining digester sludge chemical and physical uniformity throughout the tank
Rapid dispersion of metabolic wastes (products) produced during substrate digestion
Rapid dispersion of any toxic materials entering the tank (minimizing toxicity)
Prevent deposition of grit

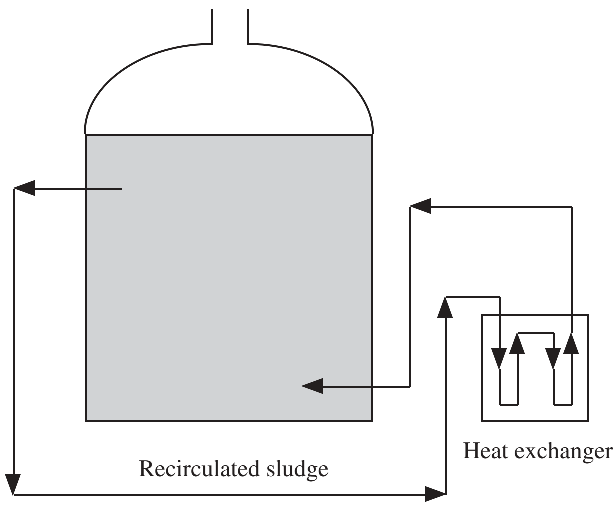


Figure 18.1

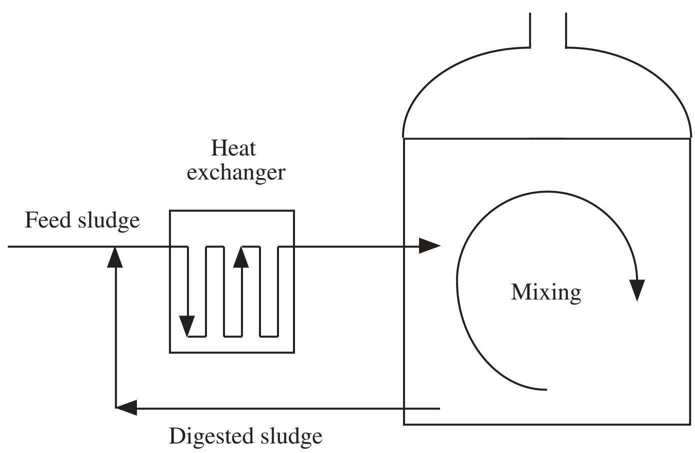


Figure 18.2

Sludge recirculation can be used for mixing digester content, but this method generally is not used. When the method is used, sludge is recirculated through heat exchangers and modest mixing is achieved. Sludge recirculation often is used when no mixing equipment is available.

Mixing need not be continuous to achieve acceptable volatile solids destruction. Continuous mixing is costly and requires a facility that will enhance the separation of digested solids from the liquid phase. Routine mixing of digester content, for example, three to six periods of mixing per day of 1- to 3-hour duration for each mixing period, may be an efficient alternate to continuous mixing.

Methane-forming bacteria are very sensitive to rapid mixing. If rapid mixing continuously washes out methane-forming bacteria in the effluent, then retention periods of <7 days are not realistic.

Part IV

Process Control and Troubleshooting

19

Upsets and Unstable Digesters

Under steady-state conditions the anaerobic digester operates without difficulty. Adequate mixing and proper, uniform temperature contribute to a steady-state condition. However, interruptions of this condition do occur, resulting in upsets and unstable digesters.

Seven basic conditions are responsible for upsets or unstable anaerobic digesters (Table 19.1). Many of the conditions are directly or indirectly related and include hydraulic overload, organic overload, pH changes, temperature fluctuations, toxicity, large withdrawal of sludge, and sudden changes. Air contamination (presence of oxygen) is possible.

There are several indicators of unstable anaerobic digesters (Table 19.2). These indicators are either increases or decreases in specific operational values. The indicators include decreases in biogas production and methane production, decreases in alkalinity and pH, decrease in volatile solids destruction, and increases in volatile acid concentration and percent carbon dioxide in the biogas.

With respect to indicators of unstable digesters, several comments are worth noting. Biogas production is not as meaningful as methane production, because only methane production represents final degradation of organic compounds. Although a decrease in methane production is associated with an unstable digester, a decrease in methane production also may be associated with a change (less substrate) in the composition of the feed sludge.

Methane production and alkalinity may be correlated, and this correlation may be used as an indicator of an unstable digester. A decrease in methane production and a decrease in alkalinity indicate toxicity occurring in methane-forming bacteria. A decrease in methane production and no significant change in alkalinity indicate toxicity occurring in methane-forming bacteria and acid-forming bacteria. An

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TABLE 19.1 Conditions Responsible for Upsets and Unstable Anaerobic Digesters

Condition	Example
Hydraulic overload	Overpumping of dilute feed sludge
Organic overload	Overpumping of concentrated feed sludge
pH changes	Drop in pH (<6.8) and loss of alkalinity
Temperature fluctuations	Overpumping of feed sludge
Toxicity	Specific inorganic and organic wastes
Large withdrawal of sludge	Excess withdrawal of sludge and reduced retention time
Sudden changes	Rapid increase in nitrate ion concentration

TABLE 19.2 Indicators of Unstable Anaerobic Digesters

Indicator	Decrease	Increase
Biogas production	X	
Methane production	X	
Alkalinity	X	
pH	X	
Volatile solids destruction	X	
Volatile acid concentration		X
Percent CO_2 in biogas		X

increase in effluent volatile solids also will take place if toxicity to both groups of bacteria occurs. However, this increase will take at least one hydraulic retention time (HRT).

HYDRAULIC OVERLOAD

Hydraulic overload is defined as occurring when HRT is reduced to a value at which the methane-forming bacteria cannot reproduce fast enough to avoid washout. Hydraulic overload may be the result of the transfer of too large a quantity of dilute sludge, sludge production exceeding digester capacity, or reduction in digester volume. Grit accumulation and scum formation contribute to decreased digester capacity. A washout of alkalinity accompanies a hydraulic overload.

The washout of alkalinity results in a loss of digester buffering capacity and the buildup of organic acids. The buildup of organic acids is commonly referred to as a "sour" digester. Neutralizing some of the acids with alkali or caustic compounds may accelerate recovery of a sour digester.

Additional concerns related to a hydraulic overload include:

- Increased heating requirements
- Increased sludge dewatering and disposal costs
- Decreased methane production
- Decreased volatile solids destruction.

Dilute raw sludges are produced through several operational conditions. The sludges may be the result of clarifier design, sludge removal equipment, and sludge

pumping schedules, especially in warm wastewater temperatures. Concerns that are related to the production of malodors during warm wastewater temperatures often dictate that dilute sludges be pumped to the digester before adequate thickening can occur.

ORGANIC OVERLOAD

An organic overload is usually accompanied by a relatively high concentration of nitrogenous wastes in municipal wastewater treatment plants. The release of ammonia during the degradation of the nitrogenous wastes may result in ammonia toxicity.

20

Foam and Scum Production and Accumulation

The production and accumulation of foam is a common problem experienced by many anaerobic digesters. Foam production is caused by several operational conditions (Table 20.1). Foam first appears in the annular space between the floating cover and the digester wall and may completely coat the floating cover and spill over the coping of the digester wall. Foam presents safety, housekeeping, and malodor concerns as well as maintenance and operational problems.

Operational problems associated with foam production and accumulation include reduced sludge feed pumping and inversion of digester solids profile, that is, thick solids are located at the top of the digester and dilute solids are located at the bottom of the digester. Maintenance problems associated with foam production and accumulation include fouling of gas collection compressors and recirculating pipes and gas binding of sludge recirculating pumps.

Foam occurs when gas bubbles become entrapped in a liquid matrix. Gases commonly associated with anaerobic digester foam include carbon dioxide, hydrogen sulfide, methane, and nitrogen. Foaming occurs because the surface tension of the liquid or sludge is reduced, resulting in the accumulation of solids over entrapped gas bubbles. Solids within the foam usually are 2–5% by weight, and the specific gravity of the foam is <1.0.

Adequate mixing must be ensured throughout the digester to reduce the amount of entrapped gases and to homogenize digester contents. Inadequate mixing may result in stratification of digester contents and insufficient stripping of gases produced during fermentation of wastes. Pockets of undigested and stratified wastes near the surface of the digester generate volatile acids, resulting in the production and accumulation of foam.

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TABLE 20.1 Operational Conditions Associated with Foam Production

Condition	Contributing Factor
Alkalinity increase	Lysis of large numbers of strict aerobic bacteria including Nocardioforms
	High percentage of activated sludge feed
Carbon dioxide increase	Change in digester fermentation reactions
Fatty acid increase	Excess grease
	Excess triglycerides
	Lysis of large numbers of strict aerobic bacteria including Nocardioforms
Mixing	Insufficient stripping of gases
	Excessive entrapment of gas from fine bubble mixing
Polymers	Excess cationic polymers from dewatering units
	Excess cationic polymers from thickening units
Solids, fine	Excessive particulate surfactants
Solids, total	Low level of total solids
Temperature fluctuations	Intermittent feeding of sludge
	Slug feeding of sludge
Scum	Rapid breakdown of scum in mature digester
Feed sludge	High organic content

Vigorous mixing or excessively high mixing rates and fine bubble gas mixing enhance the entrapment of gas and the production of foam. Coarse bubble gas mixing and mechanical mixing do not enhance the production of foam as much as fine bubble gas mixing.

Foaming episodes usually occur in anaerobic digesters during start-up, system imbalance, and overloading. Common operational conditions associated with foaming include changes in loading or concentration of alkalinity and fatty acids, cationic polymers, carbon dioxide, temperature, fine solids, and low total solids. Foaming is usually worse shortly after sludge feeding or overloading of the digester.

START-UP

During start-up, the volatile acid-forming bacteria are numerous and very active whereas the methane-forming bacteria are just becoming established. The difference in the relative abundance and activity of these two bacterial groups is a result of the short generation time of the volatile acid-forming bacteria compared with the long generation time of the methane-forming bacteria. Because of the large quantity of volatile acids present during start-up and the resulting reduction in surface tension of the sludge, carbon dioxide and methane released from the fermentation of organic wastes become entrapped in the sludge. This results in foam production. The foam is usually a light black froth that dissipates as the concentration of volatile acids decreases. Foam production in a mature digester is usually thick and black.

ALKALINITY AND FATTY ACIDS

Alkalinity is inversely proportional to surface tension, that is, as alkalinity increases within digester sludge the surface tension of the sludge decreases. The sludge becomes more surface active and has a greater propensity to foam. Increasing

alkalinity also may serve as an indicator of other operational factors that contribute to foam production. High alkalinity changes the surface tension of anaerobic digester sludge in a similar fashion as biosurfactants from dead Nocardioforms change the surface tension of activated sludge.

Alkalinity within an anaerobic digester increases because of significant changes in specific operational conditions. Operational conditions that result in an increase in alkalinity include increased alkalinity loading (ammonium ions, amino acids, proteins, and cationic polymers), death of large numbers of strict aerobic bacteria resulting in the release of large quantities of amines, and decreased alkalinity destruction within the digester.

Because wasteactivated sludge contains alkalinity (ammonium ions, amino acids, and proteins) and increases the alkalinity of the digester sludge, the alkalinity of the activated sludge should be closely monitored and regulated to control digester foam. This is especially true in warm wastewater temperatures, when increased bacterial activity in the activated sludge results in the release of ammonium ions from nitrogenous wastes. An increase in alkalinity also may serve as an indicator of an adverse operational condition, for example, change in wastewater composition.

Excess fatty acids within an anaerobic digester enhance foam production. Fatty acids are surfactants and decrease the surface tension of the sludge. Again, the reduced surface tension of the sludge results in foam production. The presence of excess fatty acids is usually associated with grease or animal fat (triglycerides) and the death of large numbers of bacteria. Phospholipids also released after the death of bacteria serve as surface-active agents that favor foam production.

Excess grease transferred to an anaerobic digester presents two significant operational problems. First, the quantity of grease may increase the solids loading rate to the digester and may adversely affect retention time. Second, the degradation of grease may result in an increase in volatile fatty acids. The fatty acids would negatively impact the buffering capacity, pH, and methane gas production of the digester.

Grease may be removed upstream of the digester and treated aerobically with appropriate bioaugmentation products. Bioaugmentation products also may have some value in the control of scum blankets and accelerating the recovery of an anaerobic digester that has experienced an upset condition.

CARBON DIOXIDE

Carbon dioxide (CO_2) is one of several gases found in foam. With increased carbon dioxide production in an anaerobic digester, the amount of carbon dioxide within the sludge also increases. An increase in carbon dioxide within the sludge promotes foam production.

Carbon dioxide content within the digester can be reduced by bubbling digester gas through a potassium hydroxide (KOH) solution or introducing natural gas into the gas system to dilute the carbon dioxide content. A decrease in carbon dioxide content results in an increase in digester pH and a more favorable volatile acid-to-alkalinity ratio.

POLYMERS

Cationic polymers used upstream of an anaerobic digester for sludge thickening and cationic polymers found in centrates and filtrates from sludge dewatering units have been suspected in the production of foam. Cationic polyacrylamide polymers contain numerous amino groups that are released as the polymers are degraded. Once released, the amino groups form ammonium ions that increase sludge alkalinity. The presence of additional ammonium ions and increased alkalinity within the sludge change the surface tension of the sludge, resulting in foam production.

SOLIDS, FINE AND LOW TOTAL

The accumulation of fine solids in the digester often is associated with foam production. Accumulation of fine solids may be due to the presence of particulate surfactants found in centrates, filtrates, and supernatants. The presence of low total solids in the digester reduces the surface tension of the sludge, resulting in the production of foam.

STRUVITE

Struvite is a cottony-white substance that mimics foam and is sometimes produced in anaerobic digesters. This substance is magnesium ammonium phosphate ($MgNH_4PO_4$).

TEMPERATURE

Fluctuations in digester temperature significantly affect the activity of volatile acid-forming bacteria and methane-forming bacteria and the concentration of products formed by the bacteria. The production of foam may occur with temperature fluctuations as small as 2°C. Slug feeding and intermittent feeding may cause temperature fluctuations.

Foaming episodes as affected by temperature fluctuations occur more frequently in thermophilic digesters than in mesophilic digesters. Because of a higher bacterial activity and the die-off of large numbers of bacteria, thermophilic digesters have higher concentrations of alkalinity and volatile acids.

SCUM

Digester scum consists of floating materials such as grease and vegetable matter with a specific gravity <1.0. Plastics, hair, and rubber products are commonly found in scum.

Scum may have entrapped bubbles of gas. If scum is broken or dissipated by mixing over a very short period of time, the rapid breakdown of the grease and vegetable matter results in a buildup of volatile acids. These acids at the surface of the digester are responsible for the production of foam.

TABLE 20.2 Control Measures for Foam and Scum Production

Measure	Description
Activated sludge	Feed sludge to one digester at a relatively low feed rate, e.g., <0.05 lb VS/ft^3/day
Digester foam/scum	Manually remove foam and scum
	Treat foam and scum with an appropriate defoaming agent
	Break bubbles by passing foam through impeller
Mixing	Produce homogenized sludge, i.e., prevent stratification of solids
Primary clarifier scum	Do not waste to digester, i.e., find alternate means of disposal
	Treat with bioaugmentation products or enzymatic products to degrade lipids
Solids loading	Avoid high or slug loadings
	Avoid intermittent loadings
	If possible, feed continuously
Temperature	Maintain stable temperature
	Avoid temperature fluctuations >2°C

Blankets of scum may form during a start-up or in a mature system. During start-up a thin zone of high volatile acid content may become localized at the digester surface. Under a mature system a thick, black layer of grease, vegetable matter, and concentrated activated sludge may cover the entire surface.

Under a mature system the breakdown of scum at the surface of the digester may cause foaming. When scum degrades, a pocket of high volatile acid content develops. The acids produce a condition that is similar to start-up. Stratification of non-digested solids near the surface causes foaming.

If foam and scum production in an anaerobic digester is a severe and frequent problem, numerous control measures are available (Table 20.2). These measures address activated sludge feed, foam and scum accumulation, adequate mixing, primary clarifier scum, solids loading to the digester, and temperature control.

Often a combination of control measures rather than just one measure may be needed to control foam and scum production and accumulation. It should be noted that the most effective measures to control foam production might not be the most effective measures to control scum production and vice versa.

21

Supernatant

When sludge is allowed to settle in a digester, a supernatant develops. Anaerobic digester supernatant is commonly returned to the head of wastewater treatment plants and mixed with the influent (Figure 21.1). Although the supernatant is relatively small in volume, it contains dissolved and suspended organic and inorganic materials. These materials add suspended solids, nutrients (nitrogen and phosphorus), and organic compounds to the influent.

The returned materials may cause a variety of operational problems (Table 21.1). Therefore, wastewater treatment plants that are not achieving significant liquid-solid separation in anaerobic digesters should consider discontinuing the practice of returning supernatant to the headworks of the plant.

Increased chlorine demand may occur because of the presence of excess ammonium ions in the supernatant. Volatile fatty acids, volatile organic compounds, volatile sulfur compounds, and hydrogen sulfide released from the supernatant in the turbulent headworks of a wastewater treatment plant may contribute to malodor problems.

Sludge bulking in the activated sludge process may occur through the weakening of floc particles by excess total dissolved solids (TDS) or the rapid and undesired growth of filamentous organisms. The presence of sulfides in the supernatant may trigger the growth of sulfide-loving filamentous organisms such as *Beggiatoa* spp. and *Thiothrix* spp., whereas the presence of readily degradable organic compounds may trigger the growth of foam-producing filamentous organisms such as *Microthrix parvicella* and Nocardioforms.

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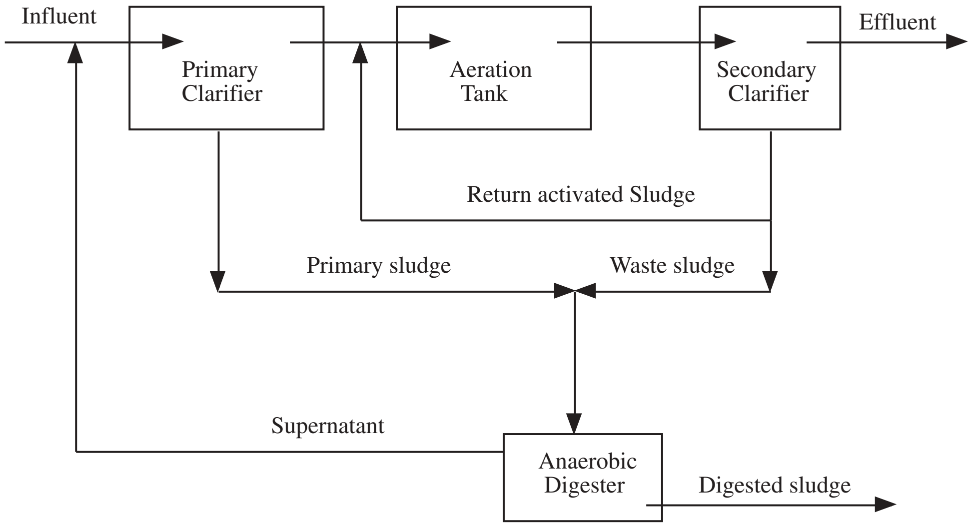


Figure 21.1

TABLE 21.1 Operational Problems Associated with the Return of Digester Supernatant to the Head of a Wastewater Treatment Plant

Increased chlorine demand
Malodor problems
Sludge bulking
Undesired impact of high concentrations of nitrogen and phosphorus

Several operational problems are associated with the presence of high concentrations of nitrogen and phosphorus. These problems include possible permit violations, nitrification, denitrification, and excess growth of algae in secondary clarifiers.

22

Monitoring

Extensive analytical monitoring of anaerobic digesters has not been a common practice at many wastewater treatment plants. Lack of adequate and timely monitoring has resulted in numerous digester failures. Lack of monitoring often is due to the relatively large amount of time required to perform the many analytical tests.

To monitor the activity of the bacteria in an anaerobic digester and evaluate and troubleshoot digester operations, several analytical tests should be performed on a periodic basis. These tests include analyses of digester content (Table 22.1) and digester feed sludge (Table 22.2). The frequencies of analyses of several common analytical tests are presented in Table 22.3. During start-up and upsets analytical tests should be performed more frequently. Because digester sludge contains a relatively large quantity of inert solids, analysis of solids is not a meaningful measure of the amount of biomass and biomass activity.

ALKALINITY

Adequate buffering capacity or alkalinity is needed in an anaerobic digester for maintenance of proper pH. Alkalinity is produced in the digester through the degradation of some wastes, for example, cationic polymers, amino acids, and proteins, and alkalinity is lost in the digester through the production of volatile acids.

Acceptable alkalinity concentrations are normally 1000–2000 mg/l in a primary digester and 1500–3000 mg/l in a secondary digester. If a deficiency in alkalinity exists, the amount of alkalinity needed can be estimated on the basis of excess volatile acids.

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TABLE 22.1 Recommended Analytical Tests for Anaerobic Digester Content

Test	√ Desired Monitoring Frequency		
	Daily	Weekly	As Needed
Alkalinity			
Ammonical-nitrogen			
Chemical oxygen demand (COD)			
Gas composition			
Gas production			
Grease			
Organic-nitrogen			
Orthophosphate-phosphorus			
pH			
Settleable solids, supernatant			
Temperature			
Total solids			
Toxicity			
Volatile acids-to-alkalinity			
Volatile solids			
Volume level			

TABLE 22.2 Recommended Analytical Tests for Anaerobic Digester Feed Sludge

Test	√ Desired Monitoring Frequency		
	Daily	Weekly	As Needed
Alkalinity			
Ammonical-nitrogen			
Chemical oxygen demand (COD)			
Grease			
Organic-nitrogen			
pH			
Total solids			
Volatile acids			
Volatile solids			
Volume, gallons			

TABLE 22.3 Frequencies of Analyses of Several Anaerobic Digester Content Tests

Test	Frequency	Sample Type
Alkalinity	1 time/week	Composite
Ammonical-nitrogen	1 time/week	Composite
Chemical oxygen demand (COD)	1 time/day	Composite
Micronutrients	1 time/month	Composite
pH	1 time/day	Composite
Orthophosphate-phosphorus	1 time/week	Composite
Volatile acids	1–2 times/week	Composite

Alkalinity may be added to a digester through chemical addition or changes in operational measures. These measures include transferring secondary digester alkalinity to the primary digester, increasing mixing and heating times, and decreasing the amount of primary and secondary sludges that is wasted to the digester.

BIOGAS/METHANE PRODUCTION

Gas production, especially methane, increases with increasing organic loading to the digester until methane-forming bacteria are no longer capable of degrading volatile acids. The volume, rate, and composition of the biogas produced are indicative of digester performance. An acceptable or normal range of biogas production is 10–25 ft^3/lb volatile suspended solids (VSS) destroyed or 0.4–0.6 l/g of chemical oxygen demand (COD) converted at 35°C. A decrease in volume of biogas, rate of biogas production, or percent methane composition is an early indicator of digester failure.

Treatability of wastes or substrate by anaerobic digesters is usually determined by monitoring biogas production. The rate and volume of methane produced during anaerobic digestion of a waste can be used to determine its relative rate of conversion. The more rapid and the larger quantity of biogas produced, the more easily the waste is treated in an aerobic digester.

When volatile acid production occurs more rapidly than volatile acid consumption, that is, methane production, an upset condition occurs in an anaerobic digester. The digester becomes acidic or "sour." Because methane-forming bacteria are very sensitive to acidic conditions, methane production decreases as volatile acid concentration increases. Methane production usually terminates when the digester pH drops below 6.0.

pH

The pH of an anaerobic digester is mostly the result of the volatile acid-to-alkalinity ratio, but the pH is usually the last indicator to change when a digester is upset. Adjusting the volatile acid-to-alkalinity ratio or adding alkalinity impacts digester pH.

An acceptable range of pH values for a primary digester is 6.6 to 7.0. An acceptable range of pH values for a secondary digester is 6.8 to 7.2.

TEMPERATURE

Change in temperature has the most significant impact on the activity of anaerobes and the efficiency of digester operation. Change in temperature also affects the quality and quantity of products obtained through fermentation. These products may or may not be readily available substrates for methane-forming bacteria. Therefore, a change in temperature >2°C per day should not be permitted and the temperature throughout the digester should be consistent. An acceptable range of temperatures for mesophilic digesters is 30–35°C.

SETTLEABLE SOLIDS—SUPERNATANT

The characteristics of digester supernatant vary greatly according to the type of sludge feed to the digester and the type of digester used. Solids in the supernatant that are discharged to the head of the treatment plant represent particulate organic loading and solids loading on the primary clarifier and secondary treatment process. To maintain low loadings, the settleable solids in the supernatant should be <50 ml after 4–5 hours of testing. Low levels of loading may be obtained by ensuring proper digester operation and maximum settling time.

TOTAL SOLIDS—SUPERNATANT

Total solids within the supernatant that are discharged to the head of the plant also represent particulate organic and solids loadings. Total solids <5000 mg/l are acceptable. However, supernating should begin when solids are 2000 mg/l. Ensuring proper digester operation and maximum settling time also may reduce total solids in the supernatant.

TOTAL SOLIDS—SLUDGE FEED

Total solids should be 1.5–3.0% in primary sludge and 4.0–8.0% in secondary sludge. Heavy solids or solids greater than 3.0% in primary sludge may be reduced by decreasing retention time in primary clarifiers. Thin solids or solids <4.0% in secondary sludge may be increased by extending wasting intervals or adding primary sludge.

VOLATILE ACIDS

An increase in volatile acid concentration without an increase in alkalinity is an indicator of an adverse operational condition within an anaerobic digester. Acetate is a precursor for most of the methane produced in an anaerobic digester.

Butyrate and propionate are important intermediates or precursors of methane production. The accumulation of these acids or an increase in volatile acid concentration can be associated with digester instability or stress.

Acceptable volatile acid concentrations are usually 50–200 mg/l in a primary digester and 50–500 mg/l in a secondary digester. An increase in volatile acid production or decrease in pH or alkalinity usually is caused by a change in bacterial activity, that is, an increase in the activity of volatile acid-forming bacteria or a decrease in activity of methane-forming bacteria. Optimizing mixing, maintaining proper pH/alkalinity and temperature, and ensuring acceptable sludge feed and withdrawal rates promote the activity of methane-forming bacteria.

VOLATILE ACID-TO-ALKALINITY RATIO

The range of acceptable volatile acid-to-alkalinity ratios is 0.1–0.2. An acceptable ratio may be obtained by adjusting volatile acid concentration, alkalinity concentration, or both concentrations. Reducing or terminating feed sludge to the digester also helps to lower the volatile acid-to-alkalinity ratio. If feed sludge cannot be reduced or terminated, the use of chemicals for alkalinity adjustment is required.

An unacceptable volatile acid-to-alkalinity ratio is usually the first warning of an adverse operational condition. Significant deviations from an acceptable ratio may be caused by shock loading or excess sludge withdrawal

The volatile acid-to-alkalinity ratio is a key control parameter. Perhaps the best method of maintaining a properly operating anaerobic digester is to ensure an acceptable volatile acid-to-alkalinity ratio. A ratio of 0.07–0.08 is a good working ratio, whereas a ratio >0.5 is indicative of digester upset and possible failure.

Part V

Digesters

23

Types of Anaerobic Digesters

Anaerobic digesters are capable of treating insoluble wastes and soluble wastewaters. Insoluble wastes such as particulate and colloidal organics are considered to be high-strength wastes and require lengthy digestion periods for hydrolysis and solubilization. Digester retention times of at least 10–20 days are typical for high-strength wastes.

High-rate anaerobic digesters are used for the treatment of soluble wastewaters. Because these wastewaters do not require hydrolysis and solubilization of wastes, much faster rates of treatment are obtained. High-rate anaerobic digesters usually have retention times of less than 8 hours.

High-strength wastes are usually treated in suspended growth systems, whereas soluble wastewaters are usually treated in fixed-film systems. Several anaerobic digester processes and configurations are available for the treatment of insoluble wastes and soluble wastewaters (Table 23.1). Each configuration impacts solids retention time (SRT) and hydraulic retention time (HRT). Minimal HRT is desired to reduce digester volume and capital costs. Maximal SRT is desired to achieve process stability and minimal sludge production.

BACTERIAL GROWTH—SUSPENDED

In suspended growth systems, the bacteria are suspended in the digester through intermittent or continuous mixing action (Figure 23.1). The mixing action distributes the bacteria or biomass throughout the digester.

Because completely mixed anaerobic digesters do not incorporate a means for retaining and concentrating the biomass, the SRT is the same as the HRT.

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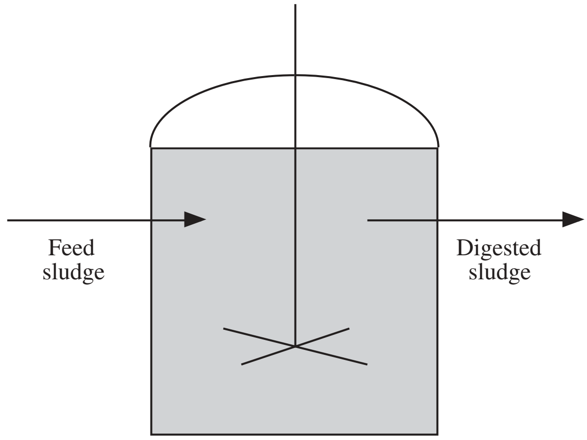


Figure 23.1

TABLE 23.1 Types of Anaerobic Digesters

Characteristic	Application
Bacterial growth system	Suspended
	Fixed film
Temperature	Psychrophilic
	Mesophilic
	Thermophilic
Configuration	Single-stage (phase)
	Two-stage (phase)

TABLE 23.2 Advantages and Disadvantages of Suspended Growth Anaerobic Digesters

Advantages	Suitable for the treatment of particulate, colloidal, and soluble wastes
	Toxic wastes may be diluted
	Uniform distribution of nutrients, pH, substrate, and temperature
Disadvantages	Large digester volume required to provide necessary SRT
	Treatment efficiency may be reduced due to loss of particulate and colloidal wastes and bacteria in digester effluent

Completely mixed anaerobic digesters are designed for relatively long HRTs. Feed sludge can be added to the digester on a continuous or intermittent basis. Advantages and disadvantages of completely mixed suspended growth digesters are listed in Table 23.2.

BACTERIAL GROWTH—FIXED FILM

Anaerobic fixed-film (sludge blankets) systems provide a quiescent environment for the growth of an agglutinated mass of bacteria (Figure 23.2). Because bacterial growth requires relatively long periods of time to develop, the media used in

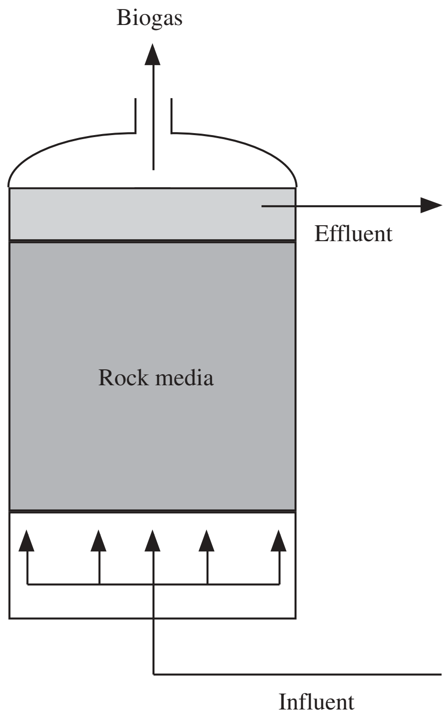


Figure 23.2

fixed-film systems hold the bacteria in the digester for relatively long periods and provide for long SRTs and short HRTs.

The bacteria grow as fixed films of dendritic or “stringlike” masses on the supportive media or as clumps of solids within the openings or voids of the supportive media. Fixed-film systems usually use gravel, plastic, and rock as the supportive media. The openings make up approximately 50% or more of the media.

Fixed-film systems operate as flow-through processes, that is, wastewater passes over and through a bed of fixed film of bacteria growth and through entrapped clumps of bacterial growth (Figure 23.3). Soluble organic compounds in the wastewater are absorbed (diffuse into) by the bacteria, whereas insoluble organic compounds are adsorbed (attach) to the surface of the bacteria. The flow of wastewater through fixed-film systems may be from the bottom to the top (upflow) or from the top to the bottom (downflow) (Figure 23.4).

Because the bacteria (solids) in fixed-film systems remain in the digester for long SRTs, the systems allow methane-forming bacteria to acclimate to toxicants such as ammonia, sulfide, and formaldehyde. Therefore, anaerobic fixed-film systems with long SRTs and short HRTs may be used to treat industrial wastewater containing toxicants.

Numerous fixed-film systems are available for use in the digestion of municipal and industrial wastewaters and sludges (Table 23.3 and Figure 23.5). These systems are capable of treating a variety of wastewaters and sludges, provide good contact

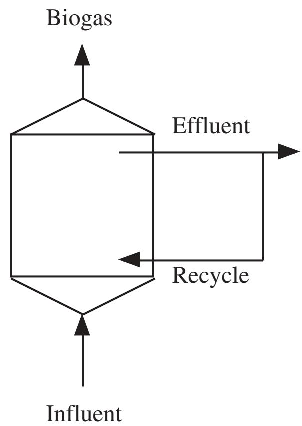


Figure 23.3

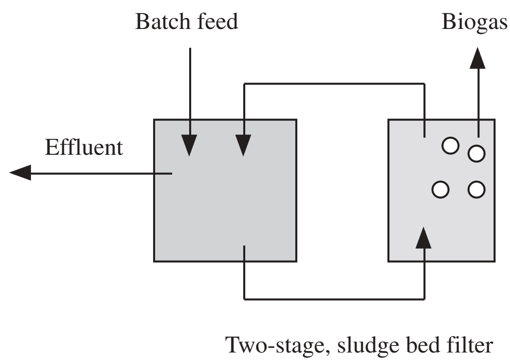


Figure 23.4

TABLE 23.3 Anaerobic Fixed-Film Systems

Baffled reactor
Expanded bed
Expanded microcarrier bed (MCB)
Fluidized-bed reactor
Fully packed upflow
Hybrid flow
Modular
Rotating biological contactor
Thin-film bioreactor
Upflow anaerobic sludge blanket (UASB)

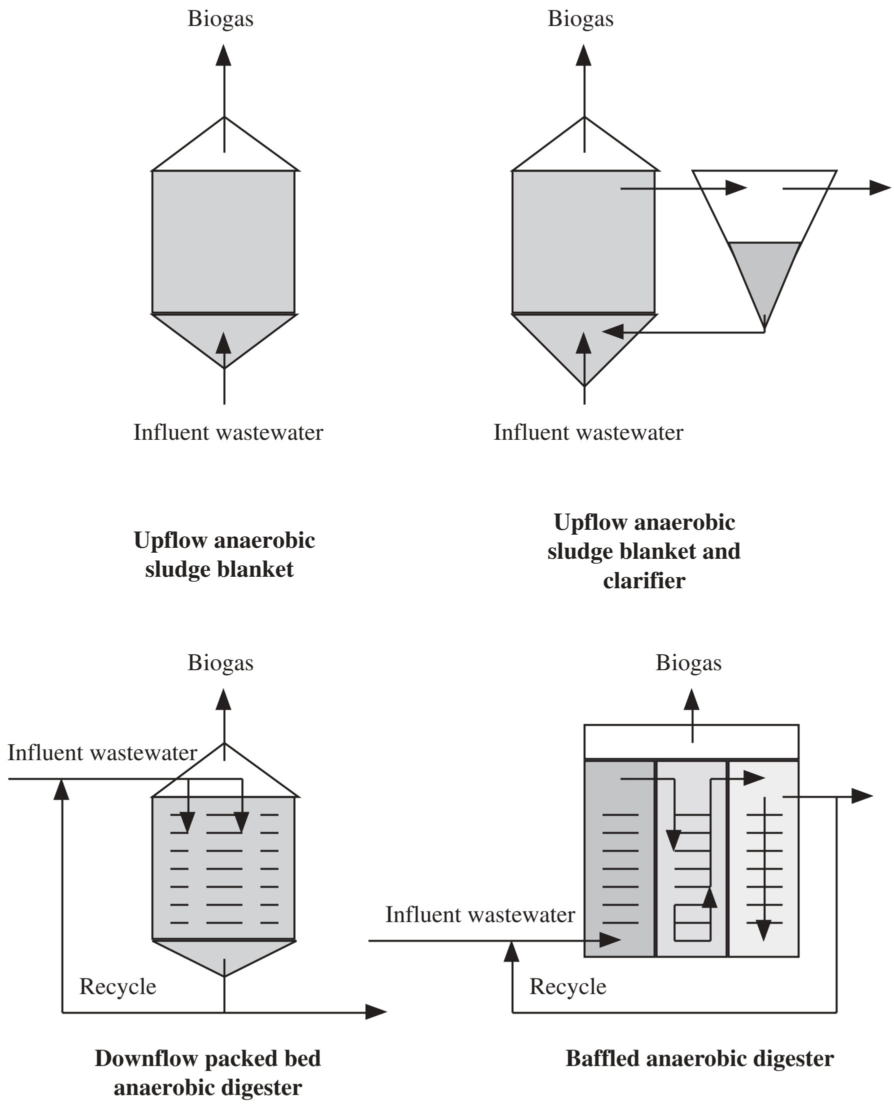


Figure 23.5

between the wastes and the bacteria, and can treat wastewaters and sludges over a relatively large range of temperature values (4–55°C) (Table 23.4).

TEMPERATURE—PSYCHROPHILIC

Psychrophilic sludge digestion and methane production occur at a relatively low temperature range (5–20°C). Because of less than optimal activity of the anaerobic bacteria in the psychrophilic temperature range, digestion of sludge is limited to small-scale operations such as Imhoff tanks, septic tanks, and sludge lagoons. The

TABLE 23.4 Examples of Wastewaters and Sludges Treated by Anaerobic Fixed-Film Digesters

Airport deicing fluids
Contaminated groundwater
Industrial wastewaters containing high concentrations of carbohydrates
Industrial wastewaters containing high concentrations of nitrogenous compounds
Low-strength wastewaters (<600 mg/l COD) at relatively short HRTs (<6 hours)

temperature of the digester contents is approximately the temperature of its surrounding environment and varies from season to season. Because temperatures in psychrophilic digesters are relatively low, the SRTs of these digesters are greater than 100 days.

TEMPERATURE—MESOPHILIC

Mesophilic sludge digestion and methane production occur at a moderate temperature range (30–35°C). Mesophilic anaerobic digestion of sludge is commonly used at municipal and industrial wastewater treatment process and offers two practical advantages of operation compared with psychrophilic and thermophilic anaerobic digestion. First, there are more anaerobic mesophiles in nature than there are psychrophiles and thermophiles. Second, it is less expensive to maintain mesophilic temperatures in digesters than it is to maintain thermophilic temperatures. Most anaerobic digesters in North America operate in the mesophilic range.

TEMPERATURE—THERMOPHILIC

Thermophilic sludge digestion and methane production occur at a high temperature (50–60°C). Thermophilic anaerobic digestion of sludge is more often used at industrial wastewater treatment plants, where process heat or steam is available to heat digesters to the thermophilic range.

Because of the high operating temperature of these digesters, sludge digestion and methane production occur rapidly and significant destruction of pathogens is achieved. However, in addition to high operation costs, thermophilic digesters do have some significant microbiological concerns with respect to their use in degrading sludges. The number of thermophilic methane-forming bacteria is very limited, the bacterial growth is slow, and the bacterial population experiences a high endogenous death rate. Also, the bacteria are very sensitive to fluctuations in digester temperature.

CONFIGURATION—SINGLE STAGE

A typical single-stage digester consists of only one tank or reactor. Digester operations consist of sludge addition and withdraw, mixing, heating, gas collecting, and supernating. These operations are possible because of stratification of the

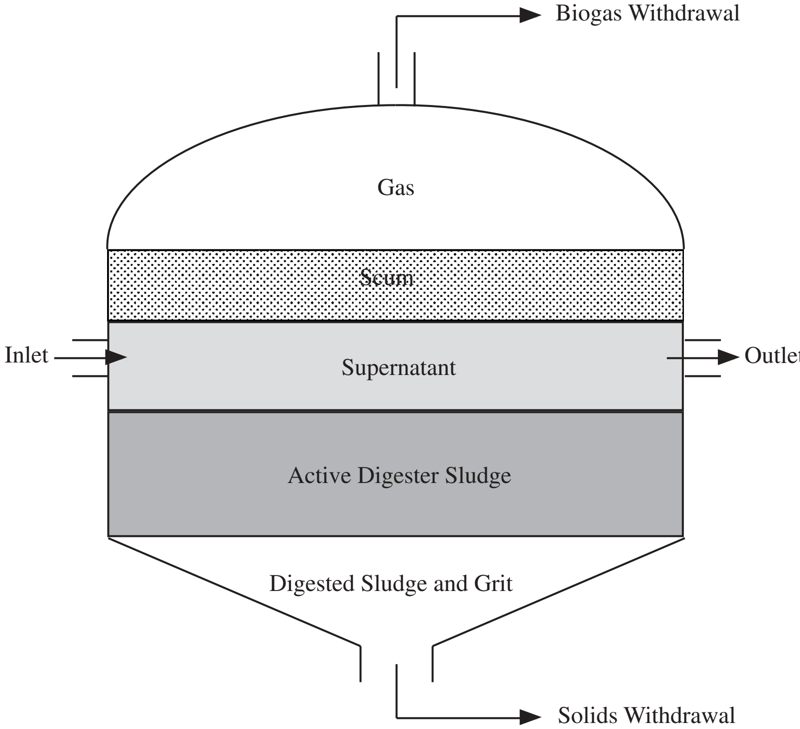


Figure 23.6

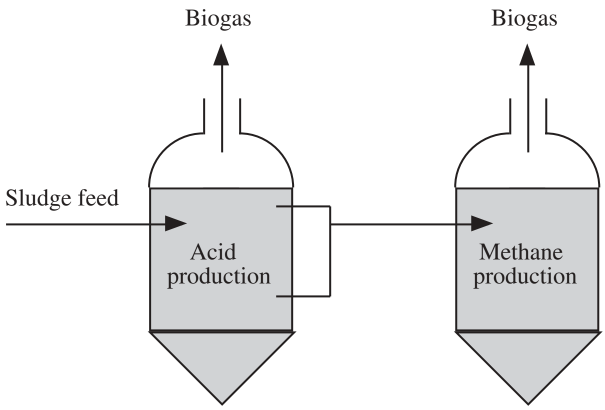


Figure 23.7

digester content. Stratification results in the following layers from top to bottom of the digester: gas, scum, supernatant, active digester sludge, and digested sludge and grit (Figure 23.6).

Single-stage digesters are more easily upset than two-stage digesters. This is because of the presence of the simultaneous activities of two groups of bacteria, the

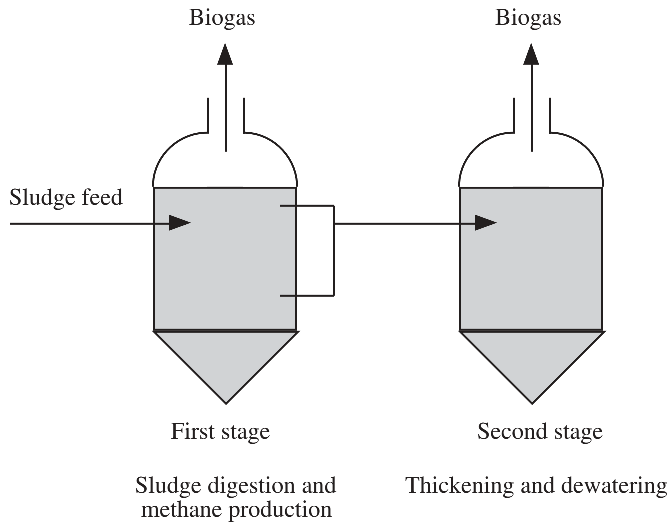


Figure 23.8

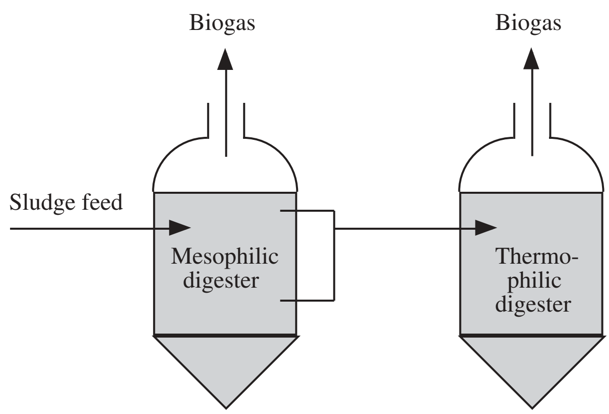


Figure 23.9

acid-forming bacteria and the methane-forming bacteria. Because acid-forming bacteria grow more quickly than methane-forming bacteria and are more tolerant of fluctuations in operational conditions, an imbalance between acid production rate and methane production rate often occurs. This imbalance may cause a decrease in alkalinity and pH that results in digester failure.

CONFIGURATION—TWO STAGE

Two-stage digester systems consist of at least two separate tanks or reactors. A limited variety of two-stage systems are available. A two-stage system yields improved efficiency and stability over a single-stage system. A two-stage system is

capable of obtaining methane production and solids reduction similar to those of a single-stage system at a smaller HRT. Also, toxicants are removed in the first stage.

In some two-stage systems acid production occurs in the first stage or tank and methane production occurs in the second stage (Figure 23.7). In some two-stage systems, sludge digestion and methane productions occur simultaneously and continuously in one tank and sludge thickening and storage occur in the other tank (Figure 23.8). In this configuration the first stage is continuously mixed and heated for sludge digestion, whereas stratification is permitted in the second stage, where sludge thickening and storage occur.

Other two-stage systems consist of temperature-phased anaerobic digestion of sludges or wastewaters. These systems are a combination of thermophilic and mesophilic anaerobic digestion (Figure 23.9). These systems provide for improved dewaterability of sludges and reduction in numbers of pathogens.

24

Anaerobic Digesters versus Aerobic Digesters

Aerobic and anaerobic digesters can degrade organic compounds. The aerobic process consists of a large variety of bacteria working side by side to degrade the organic compounds, whereas the anaerobic process consists of a large variety of bacteria working in sequence, that is, one after the other (Figure 24.1).

During aerobic degradation of organic compounds, aerobes and facultative anaerobes use free molecular oxygen to completely degrade organic compounds such as proteins to CO_2, H_2O, new bacterial cells (sludge), and inorganic compounds such as NH_4^+, HPO_4^{2-}, and SO_4^{2-} (Equation 24.1). The aerobic degradation of organic compounds is enhanced by the activity of higher life-forms—ciliated protozoa, rotifers, and free-living nematodes (Figure 24.2).

$$\text{organic compound (protein)} + O_2 \rightarrow CO_2 + H_2O + \text{cells} + NH_4^+ + HPO_4^{2-} + SO_4^{2-} \tag{24.1}$$

Nitrification occurs in the aerobic process. During nitrification strict aerobic, nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*, oxidize NH_4^+ to NO_3^- (Equations 24.2 and 24.3). The sources of ions that are oxidized in an aerobic digester include ammonium ions, amino acids, proteins, cationic polymers, and surfactants that were not oxidized or degraded in an upstream treatment process, for example, activated sludge or trickling filter.

$$NH_4^+ + 1.5O_2 \text{ — } \textit{Nitrosomonas} \rightarrow NO_2^- + 2H^+ + H_2O + \text{energy} \tag{24.2}$$

$$NO_2^- + 0.5O_2 \text{ — } \textit{Nitrobacter} \rightarrow NO_3^- + \text{energy} \tag{24.3}$$

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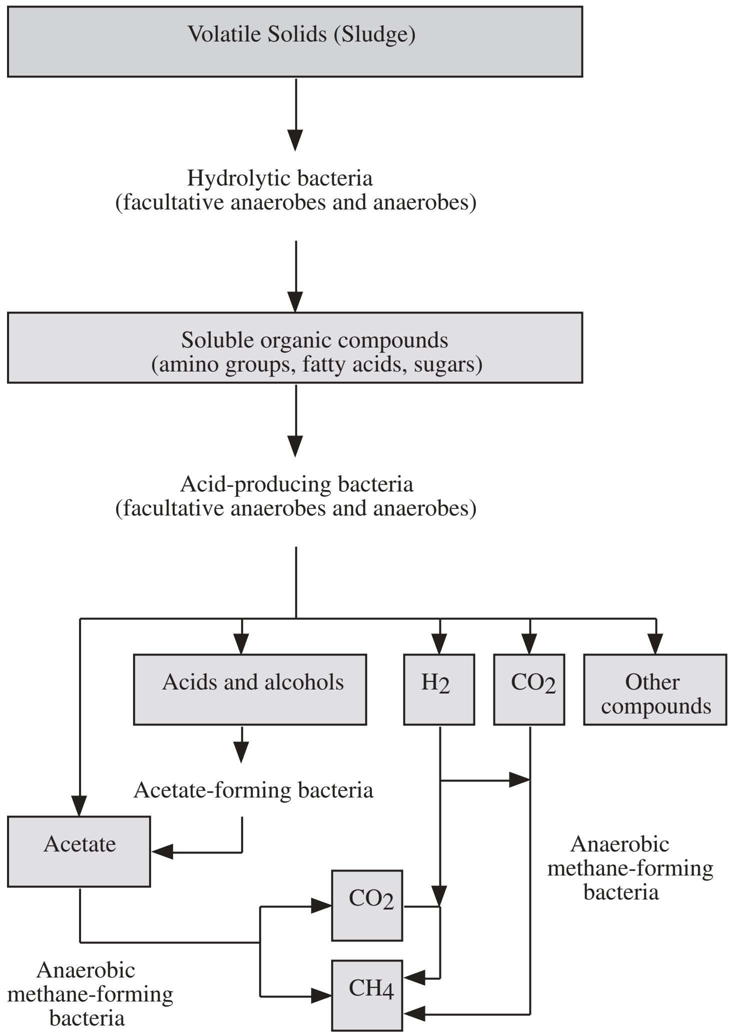


Figure 24.1

During aerobic degradation of organic compounds, the carbon from the compounds is degraded completely and is incorporated in the end products CO_2 and new bacterial cells (sludge). This is complete oxidation of the organic compounds or substrate.

Complete oxidation of organic compounds also can occur with nitrate ions (NO_3^-) instead of free molecular oxygen (Equation 24.4). If nitrate ions are used, molecular nitrogen is produced. During complete oxidation of organic compounds with nitrate ions, the carbon from the compounds is degraded completely and is incorporated in the end products CO_2 and new bacterial cells (sludge).

$$NO_3^- + 1.8CH_3OH + H^+ \rightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O \quad (24.4)$$

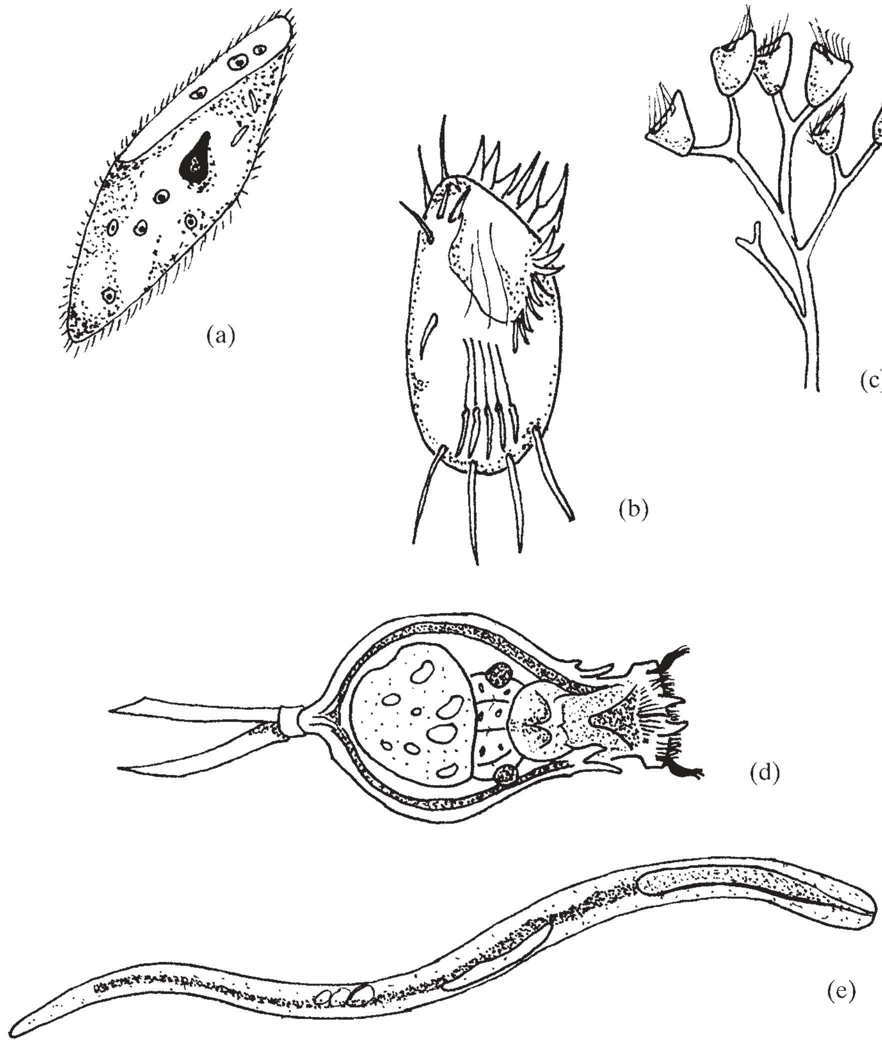


Figure 24.2 *Higher life forms in aerobic digesters. The degradation of organic wastes in aerobic digesters is enhanced through the activities of higher life forms such as the free-swimming ciliate* Paramecium *(a), the crawling ciliate* Euplotes*, the stalked ciliates* Epistylis *(c), the rotifer (d), and the free-living nematode (e).*

If free molecular oxygen or nitrate ions are not available to the aerobic process, nitrification stops and facultative anaerobes and aerotolerant anaerobes degrade the organic compounds. The organic compounds such as proteins then are degraded through fermentative reactions to CO_2, H_2O, new bacterial cells, inorganic compounds including hydrogen, and a variety of smaller compounds such as organic acids and alcohols (Equation 24.5). During anaerobic degradation of organic compounds in an aerobic digester that has no free molecular oxygen or nitrate ions, all of the carbon in the organic compounds is not degraded completely. Although some of the carbon is incorporated in the end products CO_2 and new bacterial

cells (sludge), some of the carbon remains in the end products organic acids and alcohols.

$$\text{organic compound (protein)} \xrightarrow{\text{fermentation}} CO_2 + H_2O + \text{cells} + NH_4^+ + HPO_4^{2-} + H_2S + \text{organic acids and alcohols} \quad (24.5)$$

These fermentative reactions result in incomplete oxidation of the organic compounds because some, often much, of the carbon in the degraded organic compounds is not incorporated in CO_2 and new bacterial cells. Some of the carbon is incorporated in the fermentative products, organic acids and alcohols. These products still contain much energy. These products cannot be degraded further to methane because the strict anaerobic methane-forming bacteria were destroyed in the presence of free molecular oxygen in the aerobic digester.

The degradation of organic compounds in anaerobic digesters is not enhanced by the activity of ciliated protozoa, rotifers, and free-living nematodes. Anaerobic protozoa usually are not found in large numbers in anaerobic digesters, and rotifers and free-living nematodes are strict aerobes that die in an anaerobic digester.

Nitrogenous wastes in an anaerobic digester consist of ammonium ions, amino acids, proteins, cationic polymers, and surfactants that were not degraded upstream of the digester. Another component of the nitrogenous wastes in anaerobic digesters is the nitrogen-containing compounds released by dead bacteria and higher life-forms. Strict aerobic bacteria including *Nitrosomonas* and *Nitrobacter* die in the absence of free molecular oxygen. Because of the die-off of these two genera of nitrifying bacteria, nitrogenous wastes cannot be nitrified in an anaerobic digester, that is nitrite ions (NO_2^-) and nitrate ions cannot be produced.

There are significant microbiological (Table 24.1) and operational differences between the degradation of organic compounds by aerobic and anaerobic digesters. Microbiological differences include the types of bacteria involved in the degradation process, the final electron carrier of degraded compounds, the quantity of new bacterial cells or sludge produced, and the products obtained from the degradation process.

TABLE 24.1 Significant Microbiological Differences Between Aerobic and Anaerobic Digesters

Microbiological Feature	Aerobic Digester	Anaerobic Digester
Significant bacteria	Strict aerobic, including nitrifying bacteria Facultative anaerobic	Facultative anaerobic, anaerobic, including methane-forming
Final electron carrier	Free molecular oxygen	Organic compounds, hydrogen, sulfur compounds, carbon dioxide
Number of cells produced	Higher	Fewer
Products from reactions	CO_2, H_2O, cells, NH_4^+, NO_3^-, SO_4^{2-}, HPO_4^-	CO_2, H_2O, cells, NH_4^+, CH_4, H_2, H_2S
Higher life forms	Numerous, ciliated protozoa, metazoa	Few, ciliated protozoa
Nitrification	Yes	No

TABLE 24.2 Advantages and Disadvantages of Aerobic and Anaerobic Digesters

Feature	Digester	
	Aerobic	Anaerobic
Alkalinity additional	If nitrifying	Yes
Degradation rate of organics	Rapid	Slow
Degradation of xenobiotics	No	Yes
Design and construction costs	Higher	Lower
Heating requirement	No	Yes
Malodor production	Yes	No
Methane production	No	Yes
Nutrient requirements	Higher	Lower
Operating costs	Higher	Lower
Oxygen requirement	Yes	No
Pathogen destruction	Less	More
Sensitivity to changes	Less	More
Sludge disposal costs	Higher	Lower
Sludge production	Higher	Lower
Solids retention time	Lower	Higher
Start-up time	Lower	Higher

Operational differences between aerobic and anaerobic digesters include the types and strengths of sludge or wastewaters that can be treated, start-up time, sensitivity to changes in operational conditions, operational costs, and nutrient requirements. The differences in operational conditions can be described as advantages and disadvantages (Table 24.2)

The carbon in organic compounds is used for cellular growth and reproduction by bacteria. The new cells produced from the carbon are referred to as solids or sludge. The carbon within organic compounds is available for bacterial use in primary and secondary sludges. When complete oxidation of organic compounds occurs, more cells (sludge) are produced compared with incomplete oxidation of organic compounds.

Because of the relatively high solids retention time (SRT) and fermentative pathways of anaerobic digesters, properly operated anaerobic digesters are capable of achieving significant reduction in quantity of sludge and percent volatile content of sludge. The high SRT permits the solubilization and degradation of particulate and colloidal compounds. The fermentative pathways permit low cellular reproduction (sludge yield) and the degradation of organic acids and alcohols to methane, hydrogen, and carbon dioxide. A significant advantage of anaerobic digesters is the low growth rate of bacteria or synthesis of sludge. However, during start-up, toxicity, and recovery from toxicity this low growth rate is a disadvantage.

As organic compounds are degraded in an anaerobic digester more bacterial cells (sludge) are produced. However, because more of the carbon and energy in the degraded compounds goes into waste products (organic acids and alcohols) during anaerobic digestion compared with aerobic digestion, anaerobic digesters produce less sludge than aerobic digesters. Much of the carbon and energy from organic compounds that are degraded in anaerobic digesters can be found in methane. Approximately 50% of the organic carbon from degraded compounds in aerobic digesters can be found in new bacterial cells or sludge, whereas approximately 5%

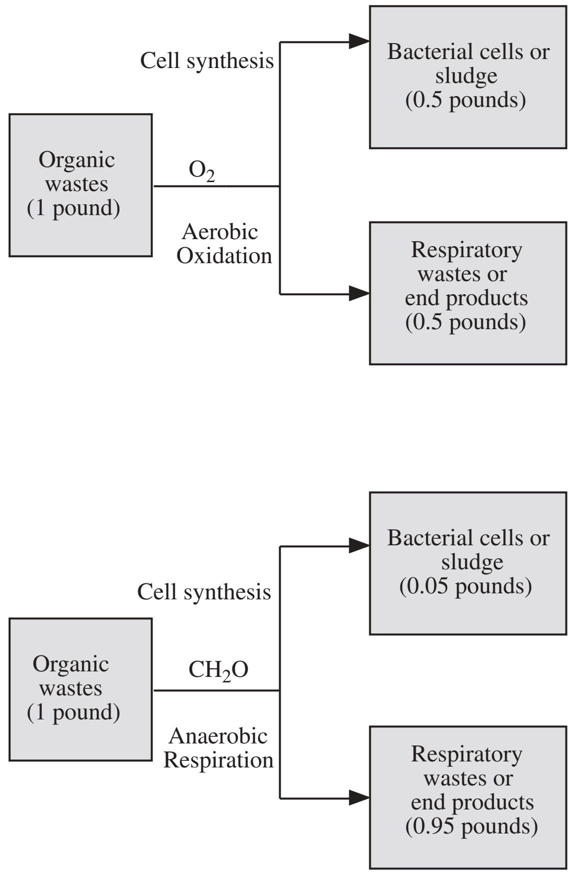


Figure 24.3 *Aerobic respiration (top) produces more bacterial cells or sludge from one pound of organic waste than does anaerobic respiration from the same pound of organic waste.*

of the organic carbon from degraded compounds in anaerobic digesters can be found in new bacterial cells or sludge (Figure 24.3).

Besides the significant difference in sludge production between aerobic and anaerobic digesters, there are other differences (advantages and disadvantages) between aerobic and anaerobic digesters (Table 24.2). Of importance is the ability of anaerobic digesters to destroy pathogens. Numerous pathogens are present in wastewaters and sludges and consequently enter digesters. Because of the high temperature and long detention time of anaerobic digesters compared with aerobic digesters, significant reduction in the number of viable pathogens occurs.

Anaerobic digester sludge that has a significant reduction in pathogens as well as malodors and reduced volatile content may be used as a soil conditioner or additive. The anaerobically digested sludge contains nitrogen and phosphorus and other nutrients that can be used to improve the fertility and texture of soils.

Another advantage of the anaerobic digester is the production of methane. This gas is a usable source of energy. The energy within methane is in excess of that

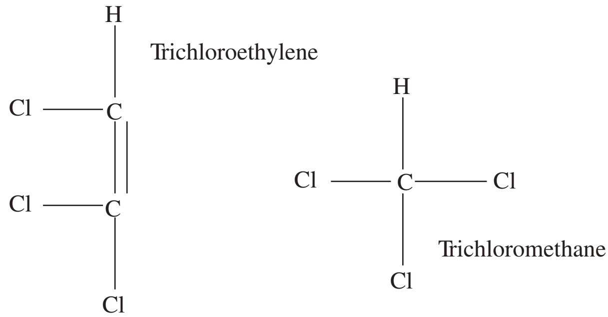


Figure 24.4

required to maintain digester temperature at most wastewater treatment plants. Methane may be used to heat the digester, heat buildings, and generate electricity.

Anaerobic digesters are capable of efficient performance over a relatively wide range of operating conditions and are capable of degrading xenobiotic compounds and recalcitrant compounds. Examples of xenobiotic compounds include chlorinated aliphatic hydrocarbons such as trichloroethylene and trihalomethanes (Figure 24.4). An example of a natural recalcitrant compound is lignin.

The principal disadvantages of anaerobic digesters include the high capital costs, the long SRTs, and the quality of the supernatant. High capital costs occur because of the need for large, covered tanks, sludge feed and circulating pumps, heating equipment, and gas mixing equipment. Long SRTs are required to grow a large and active population of methane-producing bacteria. The quality of the digester supernatant often is poor. The supernatant may contain relatively high concentrations of suspended solids, soluble organic compounds, and nutrients (nitrogen and phosphorus).

Despite these disadvantages, there is renewed interest in the use of anaerobic digesters. As regulatory agencies require digesters to reduce the number of viable pathogens significantly and produce more stable and less odorous sludges, a variety of anaerobic digesters are being used to satisfy these requirements.

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Abbreviations and Acronyms

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BOD	Biochemical oxygen demand
BTU	British thermal unit
C	Celsius
COD	Chemical oxygen demand
d	Day
F	Fahrenheit
ft^3	Cubic feet
HRT	Hydraulic retention time
I/I	Inflow and infiltration
kg	Kilogram
mg/l	Milligrams per liter
MLVSS	Mixed liquor volatile suspended solids
mV	Millivolt
ORP	Oxidation-reduction potential
SRT	Solids retention time
sp.	(one) species
spp.	(two or more) species
SRB	Sulfate-reducing bacteria
TDS	Total dissolved solids
μm	Micron
VFA	Volatile fatty acids
VOC	Volatile organic compounds
VS	Volatile solids
VSC	Volatile sulfur compounds
VSS	Volatile suspended solids

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Chemical Compounds and Elements

Ca	Calcium
$CaCO_3$	Calcium carbonate
$-CH_3$	Methyl group
CH_4	Methane
$CH_3CH_2CH_2COOH$	Butyrate
CH_3CH_2OH	Ethanol
C_2H_5CHO	Butyraldehyde
$CH_3CH_2CH_2OH$	Propanol
$CH_3(CH_2)_2CH_2OH$	Butanol
$CH_3CH_2CH_2NH_2$	Propylamine
$CH_2{=}CHCH_2SH$	Allyl mercaptan
CH_3CHNH_2COOH	Alanine
CH_3CO	Acetaldehyde
CH_3CH_2COOH	Propionate
$CH_3(CH_2)_3COOH$	Valeric acid
$(CH_3)_2CHCH_2COOH$	Isovaleric acid
$CH_3C_6H_4SH$	Thiocresol
$C_6H_5CH_2SH$	Benzyl mercaptan
$CH_3(CH_2)_4COOH$	Caproic acid
$CH_3CHOHCOOH$	Lactate
CH_3COCH_3	Acetone
CH_3COOH	Acetate
C_5H_6N	Pyridine
C_9H_9N	Skatole
$C_8H_{13}N$	Indole

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$CH_3NCH_3CH_3$	Trimethylamine
CH_2O	Formaldehyde
CH_3OH	Methanol
$C_5H_{10}O_5$	Deoxyribose
$C_6H_{12}O_6$	Glucose
$C_5H_7O_2N$	Bacterial cells
CH_3NH_2	Methyl amine
$C_3H_5NH_2$	Ethylamine
CH_3NHCH_3	Dimethylamine
$(CH_3)_2S$	Dimethyl sulfide
CH_3CH	Methyl mercaptan
C_2H_5SH	Ethyl mercaptan
CN	Cyanide
Co	Cobalt
CO	Carbon monoxide
CO_2	Carbon dioxide
–C–O–C-	Acetal bond
–COOH	Carboxylic acid group
CS_2	Carbon disulfide
Cu	Copper
Fe	Iron
Fe_2S_3	Iron sulfide
H^+	Hydrogen proton
H_2	Hydrogen gas
HCOOH	Formate
HPO_4^{2-}	Orthophosphate
HS^-	Sulfide
H_2S	Hydrogen sulfide
$HSCH_2COOH$	Thioglycolic acid
K	Potassium
KOH	Potassium hydroxide
Mg	Magnesium
N_2	Molecular nitrogen
Na	Sodium
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
$-NH_2$	Amino group
NH_3	Ammonia
NH_4^+	Ammonium ion
NH_4HCO_3	Ammonium carbonate
$H_2N(CH_2)_4NH_2$	Putrescine
$H_2N(CH_2)_5NH_2$	Cadaverine
Ni	Nickel
N_2O	Nitrous oxide
NO_2^-	Nitrite ion
NO_3^-	Nitrate ion
O_2	Free molecular oxygen
OH^-	Hydroxyl ion

PbS	Lead sulfide
PO_3^{2-}	Phosphate
S	Sulfur
–SH	Thiol group
SO_4^{2-}	Sulfate ion
Zn	Zinc

Glossary

absorb Penetration of a substance into the body of an organism

aceticlastic cleavage Conversion of acetate to methane by methane-forming bacteria

acetogenesis Production of acetate by acetate-forming bacteria

acetotrophic Use of acetate by bacteria as a substrate

activator Metal or vitamin incorporated into an enzyme that improves the efficiency of enzymatic activity

acute Having a sudden onset and short course

adsorb The taking up of one substance at the surface of an organism

aerotolerant Anaerobes that can survive in the presence of free molecular oxygen

aldehyde A compound containing the CO- radical attached to both a hydrogen atom and a hydrocarbon radical, i.e., R–CHO

aliphatic Chainlike pattern of carbon units bonding together

amino acid A group of organic acids in which a hydrogen atom of the hydrocarbon (alkyl) radical is exchanged for the amino group; used in the production of proteins

anaerobic An environment in which bacteria do not use free molecular oxygen

anoxic An environment in which bacteria use nitrite ions or nitrate ions

anthropogenic Produced under the influence of human activity

bioaugmentation The addition of commercially prepared cultures of bacteria to a wastewater treatment process to improve operational conditions

biochemical A chemical reaction occurring inside a living cell

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biomass The quantity or weight of all organisms within the treatment process

biorecalcitrant A compound that is degraded slowly by organisms

biosolids Thickened and dewatered sludge obtained from a digester

biosurfactant A compound released by an organism that reduces the surface tension of wastewater or sludge and permits the production of foam

carbonaceous A compound that is organic or contains carbon and hydrogen

catabolic Destructive or degradative biochemical reactions

catalyst A substance that accelerates a chemical reaction

catechol A phenolic compound found in vegetable matter and coal tar

cellulose A polysaccharide consisting of numerous glucose molecules linked together to form an insoluble starch

centrate The liquid and its content that are discharged from a centrifuge

chronic Having a long term or duration

clostridia Anaerobes in the bacterial genus *Clostridium*

coenzyme An activator added to an enzyme

colloid Suspended solid with a large surface area that cannot be removed by sedimentation alone

consortium Many organisms grouping together in beneficial relationship

denitrification The use of nitrite or nitrate ions by facultative anaerobes to degrade substrate

desulfurication The use of sulfate ions by anaerobes to degrade substrate

disaccharide Two sugar units (mers) or monosaccharides joined together

electron A fundamental particle with negative charge; electrons are grouped around the nuclei of atoms in several possible orbits

endoenzyme An enzyme used inside the cell to degrade substrate

endogenous The degradation of internal reserve substrate

enumerate To count

eubacteria True bacteria

exoenzyme An enzyme used outside the cell to hydrolyze substrate

facultative anaerobe Bacterium capable of using free molecule oxygen or other carrier molecule to degrade substrate

fermentation A mode of energy-yielding metabolism that involves a sequence of oxidation-reduction reactions to degrade organic substrates

filtrate The liquid and its content that pass through filter paper or a belt filter press

free-living Living or moving independently

generation time The time required for the cell population or biomass to double

halophile Freshwater organisms capable of surviving in salt water

humic substance Complex organic substances occurring in soil

hydrocarbon A general term for organic compounds that contain only carbon and hydrogen

hydrolysis The biochemical process of decomposition involving the splitting of a chemical bond and the addition of water

hydrogenotroph The use of hydrogen by bacteria as a substrate

hyperthermophile Organisms that grow at very high temperatures

intermediate A compound produced during a biochemical reaction that usually is short lived; a compound that usually does not accumulate

lignin A mixture of substances produced by certain cells of plants

lipolytic An enzyme that attacks or degrades lipids

lysis To break open; namely, on the death of bacterial cells, the content of the cells is released to the environment

macromolecule A very large molecule with much surface area

macronutrient A nutrient required in a relatively large quantity by all bacteria

monosaccharide One sugar mer or unit having three to seven carbon units

mer Unit

metabolism Pertaining to cellular activity, such as the degradation of substrate

methylotrophic The use of methyl groups by bacteria as a substrate

micronutrient A nutrient required in a relatively small quantity by most bacteria

molecule Smallest part of a compound that exhibits all the chemical properties of that specific compound

morphologic Structural features

niche The role performed by an organism in its environment

nitrification The oxidation of ammonium ions to nitrite ions or the oxidation of nitrite ions to nitrate ions

Nocardioform A group of highly branched and specialized bacteria that produce viscous chocolate brown foam in the activated sludge process

obligate Required

organic Compound containing carbon and hydrogen

organic-nitrogen Compound containing carbon, hydrogen, and nitrogen

organic-sulfur Compound containing carbon, hydrogen, and sulfur

oxic An environment in which bacteria use free molecular oxygen to degrade substrate

oxidation The biological or chemical addition of oxygen to a compound or the removal of electrons from a compound

pathogenic Disease-causing

phospholipid Lipid containing phosphorus

photosynthesis Biochemical reaction performed by green plants in which carbon dioxide is fixed to form sugar

product Chemical compounds produced from the degradation of substrate

proteinaceous Containing proteins

proteolytic An enzyme that attacks or degrades proteins

psychrophile An organism that grows under cold temperatures (<20°C)

putrescibility Decomposition of plants and animals after death resulting in the production of obnoxious substances

quinone A compound derived from benzene

reduction The biological or chemical removal of oxygen from a compound or the addition of electrons to a compound

respiration The degradation of substrate; a mode of energy-yielding metabolism that requires a final electron carrier for substrate oxidation

rumen The first division of the stomach in ruminants, being an expansion of the lower end of the esophagus

saccharolytic An enzyme that attacks or degrades sugars

sarcina Small package

solubilization To place particulate or colloid materials in solution

substrate Compounds that are used by bacteria to obtain carbon and energy

supernatant The liquid above the settled solids

surfactant Soap or detergent; a compound that alters the surface tension of wastewater or sludge

thermoacidophile Organisms that grow at a high temperature and low pH

volatile Changing readily to a vapor

xenobiotic A synthetic product that is not formed by natural biosynthetic processes; a foreign substance or poison

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